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EFFECT OF BILE ON THE CLOTTING TIME OF BLOOD.

By HERBERT HAESSLER, M.D., and MARIANNE G. STEBBINS.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, January 29, 1919.)

Although it is known that jaundice tends to cause delayed clotting of blood, the cause of the delay is not known. Minot and his associates,[1] using Howell's method of recalcifying oxalated plasma, found that the coagulation time (prothrombin time of Howell) was increased in a series of jaundice cases, but did not suggest an explanation of the mechanism of the delay in coagulation. It seemed of interest, therefore, to determine whether or not bile or bile salts, which are, of course, present in the blood in jaundice, are in themselves capable of causing the increase in the coagulation time.

Effect of Bile on Clotting of Plasma.

Series 1.—Cats, under ether anesthesia, were bled from a large artery, through a paraffined cannula into paraffined 50 cc. centrifuge tubes containing 7.5 cc. of 1 per cent sodium oxalate in 0.9 per cent sodium chloride solution. The tubes were then centrifuged and the plasma was carefully pipetted off. If the plasma showed the least trace of hemolysis it was rejected. A series of flat bottom tubes, 22 mm. in diameter, was then set up, each containing 2 cc. of plasma and 0.5 cc. of an ox bile solution of varying concentration. To each of the tubes an amount of calcium chloride was added which had been previously found to produce a firm clot in the minimum time with the same plasma. The time necessary for the formation of a firm clot in each tube was recorded. Precipitation of fibrin was considered complete when a clot of such consistency was formed that the tubes could be inverted without loss of liquid. As shown in Table I, clotting time was delayed in proportion to the amount of bile present

[1] Minot, G. R., Denny, G. P., and Davis, D., *Arch. Int. Med.*, 1916, xvii, 101.

TABLE I.

2 cc. of oxalated plasma plus 0.5 cc. of bile solution, in dilution to give the final concentration indicated, were recalcified with the optimum quantity of calcium chloride solution.

Bile.	Clotting time.	Bile.	Clotting time.
per cent	*min.*	*per cent*	*min.*
9.0	No clot.	4.0	5
8.0	" "	3.3	4
7.7	" "	2.5	4
7.4	52	2.0	4
6.9	32	1.0	3
6.6	27	0.0	3
5.0	14		

in the plasma. The results were uniform with all the animals in this series.

Similar results were obtained when whole oxalated blood was used to which varying quantities of bile had been added.

Effect of Sodium Glycocholate on Clotting of Plasma.

Series 2.—In these experiments a solution of sodium glycocholate was substituted for bile. The experiments were otherwise identical with those of Series 1. Table II, the record of a typical experiment of this series, shows that results are similar to those of the first set.

We may conclude that bile and bile salts, in sufficient quantity, retard the coagulation of blood. Most modern workers conceive of the process of coagulation as taking place in two steps: first, the formation of thrombin; and second, the conversion of fibrinogen into

TABLE II.

0.5 cc. of oxalated plasma, containing the indicated concentration of glycocholate, was recalcified with the optimum quantity of calcium chloride solution.

Sodium glycocholate.	Clotting time.	Sodium glycocholate.	Clotting time.
per cent	*min.*	*per cent*	*min.*
0.5	13	0.2	5
0.4	9	0.1	4
0.3	5	0.0	5

fibrin by the action of thrombin. To determine whether the bile salts prevented the formation of thrombin or merely interfered with the change of fibrinogen to fibrin even though an adequate supply of thrombin was present, the effect of bile and bile salts on the clotting time of a solution of fibrinogen in the presence of sufficient quantities of preformed thrombin was tested in Series 3 and 4. Solutions of fibrin and thrombin were prepared according to the method of Mellanby.[2] Not only is this method simple and rapid, but the other clotting elements which are present in small amounts do not interfere.

Preparation of Fibrinogen.—Fowl plasma was used because it may be kept for weeks without spontaneous clotting. Fibrinogen was precipitated out by diluting the plasma with ten volumes of distilled water and neutralizing with a few drops of 1 per cent acetic acid. The precipitate was collected and dissolved in 0.5 per cent sodium chloride solution, about 40 cc. being a convenient amount when 50 cc. of plasma were originally used. Since this solution clotted upon addition of both thrombokinase and calcium, but not upon addition of either alone, it was concluded that it contained fibrinogen and prothrombin.

Preparation of Thrombin.—10 cc. of the above solution were coagulated by the addition of a trace of thrombokinase (extract of chick embryo) and calcium chloride to a concentration of 0.05 per cent. After removal of fibrin, the clear, residual fluid contains a quantity of thrombin proportional to the strength of the original fibrinogen-prothrombin solution, and a trace of thrombokinase. It is not essential to have thrombin free of other elements, provided that sufficient thrombin is available in solution and that there are no substances present which interfere with its action.

Effect of Bile and Bile Salts on Clotting of Fibrinogen in the Presence of Thrombin.

Series 3.—In these experiments 0.5 cc. of a solution of fibrinogen was used, to which bile had been added to the desired concentration. An excess of thrombin was then added and the clotting time noted. The result of one such experiment is recorded in Table III.

[2] Mellanby, J., *J. Physiol.*, 1917, li, 396.

Series 4.—Experiments in this series were carried out in the same way, except that sodium glycocholate was substituted for ox bile (Table IV).

TABLE III.

0.5 cc. of fibrinogen solution plus enough bile to make the indicated final concentration.

Bile.	Clotting time.	Bile.	Clotting time.
per cent	*min.*	*per cent*	*min.*
13	No clot.	6	15
11	Loose clot.	5	7
9	" "	4	2
7	21	3	2

TABLE IV.

0.5 cc. of fibrinogen solution plus sodium glycocholate.

Sodium glycocholate.	Clotting time.	Sodium glycocholate.	Clotting time.
per cent	*min.*	*per cent*	*min.*
1.2	No clot.	0.6	7
1.0	Loose clot.	0.5	4
0.8	14	0.4	3
0.7	10	0.0	4

Effect of Sodium Oleate on Clotting of Fibrinogen in the Presence of Thrombin.—A few experiments were also performed with sodium oleate instead of bile salts. Sodium oleate, which is the salt of an unsaturated fatty acid, is somewhat similar to sodium glycocholate in physical properties. Small amounts did not interfere with the clotting time; very large amounts inhibited clotting entirely; and moderate amounts caused delayed clotting. In no experiment, however, was there the same gradual increase in clotting time with increase of the salt which was observed in experiments with bile or sodium glycocholate. The superficial resemblances of the results, however, suggested that the mechanism may be the same as in the case of bile salts.

DISCUSSION.

It is evident from the above experiments that within certain limits clotting time depends on the percentage of bile present in solution and that the reaction is the same in experiments with pure solutions

of the substances concerned in coagulation, as in whole plasma. It likewise seems justifiable to conclude that bile and bile salts do not interfere with the formation of thrombin, since the prolongation of clotting time is just as great when preformed thrombin is added in ample quantity to fibrinogen solution, as when thrombin must be formed from its precursors in the presence of bile. It cannot be a question of destruction of the thrombin, as Morawitz and Bierich[3] showed that a quantity of freshly drawn blood which had been mixed with enough bile to inhibit clotting, could be caused to coagulate by merely diluting the mixture with isotonic salt solution. Consequently we must assume that it is the conversion of fibrinogen to fibrin that is interfered with rather than the formation of thrombin.

In our experiments it was found that there was a retardation of clotting, great enough to be detected by clinical methods, with amounts of bile greater than 5 per cent. We were unable to find reports in the literature stating the exact amounts of bile salts present in the blood in jaundice. Gilbert[4] states that in cases of obstructive jaundice bile pigment is present in the blood in quantities of from 0.7 to 1 gm. per liter. Bile itself contains about 1 gm. of pigment per liter. The relation of bile pigments and bile salts in the blood in jaundice has not been determined, but it would seem possible for the salt to be present in sufficient concentration to prevent clotting.

CONCLUSIONS.

1. Within certain limits the clotting time of blood, of blood plasma, and of solutions of fibrin to which bile salts have been added, is proportional to the quantity of bile present.

2. The bile interferes with the conversion of fibrinogen into fibrin and not with the formation of thrombin.

[3] Morawitz, P., and Bierich, R., *Arch. exp. Path. u. Pharmakol.*, 1907, lvi, 115.

[4] Gilbert, quoted by Wells, H. G., Chemical Pathology, Philadelphia and London, 3rd edition, 1918, 486.

A CHARACTERISTIC LOCALIZATION OF BACILLUS ABORTUS IN THE BOVINE FETAL MEMBRANES.

By THEOBALD SMITH, M.D.

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PLATES 20 TO 22.

(Received for publication, February 21, 1919.)

While making a study of the diseased membranes in cases of infectious abortion of cattle, the writer came upon a peculiar and characteristic habitat of *Bacillus abortus* Bang, in the epithelial covering of the chorion.

This layer of cells which faces the epithelial covering of the uterine mucosa and is in intimate contact with it covers the intercotyledonous areas of the chorion and is continuous with the epithelium of the villosities of the cotyledons which dip into the depressions of the maternal caruncles. The general character of the cells may be seen in Fig. 4. The cells vary somewhat in height. The vesicular nucleus is round or oval in outline and the chromatin appears as minute 1 μ spheres against the nuclear membrane. The free border of the cytoplasm appears frequently in the form of blunt finger-like or conical projections, giving the surface a fimbriated appearance. In the specific infectious disease of the fetal membranes these cells, either individually or in series, are densely filled with minute bacilli. The invasion is recognizable under a low power in that the cytoplasm of the affected cell assumes a blue color when the section is stained in eosin-methylene blue (Fig. 1). High powers resolve this tint into fine, short, rod-like bodies (Figs. 2 to 6). The bacilli do not lie on the cell or in the ectoplasm but fill the cell body entirely. When the microscope is raised or lowered the cytoplasm appears filled in all optical sections.

The host cells are more or less altered as to size and condition of nucleus. Some cells are still normal in size and the nucleus is recog-

nizable as a vesicle with contained chromatin (Fig. 4). In others the nucleus is compact, pyknotic. The cell body becomes enlarged and it may measure up to 40 μ in diameter (Fig. 6). Vacuolation of the cytoplasm is not uncommon (Fig. 3). Fetal membranes at the end of the normal period of gestation which are macroscopically without pathological changes have been found uniformly free from such cell contents. The same is true of fetal membranes from cases of abortion associated with spirilla from which *Bacillus abortus* is not obtainable, either in cultures or through guinea pig inoculation.[1] In all cases in which the invasion of the epithelial cells was detected this feature was associated with positive cultures of *Bacillus abortus*, or the characteristic guinea pig disease after inoculation, or with both tests positive. Simple cover-slip preparations from the exudate or the necrotic villi were frequently sufficient to demonstrate the presence of infected epithelia. Besides the chorionic epithelium, the epithelial cells at the margin of the cotyledons and those of the outermost villi of the latter have been found invaded (Figs. 2 and 5). The bulk of the villi though undergoing profound changes are as a rule free from nests of bacteria.

That the cell localization described above is a regular occurrence wherever *B. abortus* is active may be gathered from the scanty literature by an interpretation of certain statements made, although none of the authors to be cited made an attempt to determine the precise source of the clumps of bacilli. Thus Bang[2] in 1897 describes the bacteria in films from the fetal membranes as either free or in dense clumps which appeared to have been formed inside cells. The following statement occurs in the British report:[3] "In many places the bacilli are collected into dense groups or colonies. Some of these groups look as if they were bounded by a cell membrane and give the impression of being contained inside tissue cells."[4] It is also stated[5] that: "It is an easy matter to identify the characteristic clumps of abortion bacilli in microscopic preparations made from the uterine exudate discharged immediately before or after abortion." In the Appendix[6] we read as

[1] Smith, T., *J. Exp. Med.*, 1918, xxviii, 701.

[2] Bang, B., *Z. Thiermed.*, 1897, i, 241.

[3] Great Britain Board of Agriculture and Fisheries, Report of the departmental committee on epizootic abortion, London, 1909, pt. 1.

[4] British report,[3] p. 6.

[5] British report,[3] p. 15.

[6] British report,[3] Appendix, p. 27.

follows: ". . . . so far as the membranes and swabs are concerned a positive diagnosis was based on the presence of the specific microbe in the typical clump form because these materials were always very impure and it is not possible to identify scattered abortion bacilli in a mixture of bacteria." Zwick and Zeller[7] refer to bacilli in large numbers in uterus exudate and state that cells may be filled with them. Fig. 3 illustrates very well what these writers probably refer to in most instances. In some cases, however, they may have seen leucocytes which at times are quite numerous and densely filled with bacilli. The latter are probably set free from the detached and disintegrated epithelial cells and taken in by leucocytes.

The mode of invasion of the epithelium by bacteria manifesting no true motility but only Brownian motion may be tentatively explained by assuming that the bacteria entering the uterochorionic space by way of the blood vessels in the uterine wall adhere to the ectoplasm and are rubbed into the substance of the cell by the pressure exerted by the uterine wall on the chorion. Once in the cytoplasm the bacteria find it a favorable medium for multiplication and a protection against phagocytosis. An alternative explanation would be to ascribe ameboid activities to the chorionic epithelium, for which theory at present no adequate proof exists.

It may be maintained that the cell parasitism is not an active disease process but rather secondary to it and operating only on cells partly or wholly devitalized. This interpretation has been kept in mind in the study of the various cases. When the disease is so far advanced that expulsion of the immature fetus occurs, the pathological material may fail to furnish an unequivocal answer to the above question. In several cases, however, in which a living calf at full term had fetal membranes in the early stages of infection, and in one slaughtered cow, the appearance of the invaded cells and the irregular distribution of the infection make it probable that they had been invaded while still in a normal condition (Figs. 2, 4, and 5).

The other types of epithelia, such as those of the uterine mucosa and of the amnion, have not been found invaded. The uterine epithelium resembles in its columnar appearance that of the chorion, but the cytoplasm forms a smooth, level, uninterrupted surface. The amniotic epithelium is of the flat, squamous type.

[7] Zwick and Zeller, *Arb. k. Gsndhtsamte.*, 1913, xliii, 1.

SUMMARY.

The significance of this invasion of the chorionic epithelium from the standpoint of pathogenesis cannot be properly evaluated until a more complete history of the successive localizations of *Bacillus abortus* has been obtained. It is safe to assume that this particular cell parasitism is but one of a series of localizations and centers of multiplication in the fetal membranes although evidence points to it as perhaps the earliest stage in which the organism gains by rapid, unchecked multiplication a considerable advantage over the host. The local destruction of an epithelial covering by an infectious agent when other miscellaneous infectious agents are absent may or may not be of much importance, for it would depend on the regenerative activity of the epithelium, the tendency to the gathering of injurious transudates, and the toxic substances associated with the bacilli.

It is probable that localizations also occur in the walls of the blood vessels of the chorion. Thus far only one case of this kind has been observed. The fusiform connective tissue cells of the adventitious coat of a blood vessel 0.8 mm. in diameter were completely replaced by clumps of minute bacilli. Since there is usually a slight perivascular cell infiltration in the diseased placenta this localization may be largely responsible for the circulatory disturbances which lead to death of the fetus. The case referred to may be but a greatly exaggerated illustration of the action of *Bacillus abortus* in the walls of the blood vessels where they are too few in number at any one time to be identified. It is known,[8, 9] that in the guinea pig disease with pronounced lesions *Bacillus abortus* is demonstrated only with great difficulty because of its scarcity.

The more or less specific localization and multiplication of bacteria within cells not having phagocytic functions have thus far been demonstrated in leprosy, syphilis, and in a disease of mice recently described by Tyzzer[10] who found an active invasion of both liver cells and intestinal epithelium by a bacillus. In cells to which phagocytic powers have been ascribed the specific localization of certain bac-

[8] Smith, T., and Fabyan, M., *Centr. Bakteriol., 1te Abt., Orig.*, 1912, lxi, 549.
[9] Fabyan, M., *J. Med. Research*, 1912, xxvi, 441.
[10] Tyzzer, E. E., *J. Med. Research*, 1917–18, xxxvii, 307.

teria is well known. Thus tubercle bacilli occur within the endothelial cells of the tubercle. Leprosy bacilli have been found within a variety of cell groups. Mouse septicemia bacilli occur regularly within certain phagocytic cells of the blood.[11] Recently bacteria have been found attached to the cilia of the respiratory tract in pertussis by Mallory and Hornor,[12] in a form of guinea pig pneumonia by the writer.[13] Actual occupation of epithelial cells followed by active multiplication of the invaders and destruction of the cell has, however, been frequently demonstrated for the sporozoa. That it may occur more often among bacteria is highly probable. Rapidity of multiplication and cell destruction or invisibility or both may stand in the way of a satisfactory demonstration.

EXPLANATION OF PLATES.

PLATE 20.

FIG. 1. Placenta of Heifer 203, which gave birth to a small but apparently healthy calf. Period of gestation unknown. The epithelium covering the chorion is enlarged, irregular in outline, the cytoplasm stained blue, and the nuclei are pyknotic. The cytoplasm of all is densely packed with bacilli not recognizable at this magnification. *B. abortus* was isolated from the placenta through guinea pigs. 10 days later the calf, having scoured somewhat, was killed and *B. abortus* was isolated from small foci of bronchopneumonia through guinea pigs. × 125.

FIG. 2. Placenta, Cow 146, aborted. A marginal villus of a cotyledon cut transversely and showing two epithelial cells *in situ* densely filled with bacilli. One cell is enlarged, projecting, the other very flat, cut transversely, and showing as a slender, nematode-like body. The nucleus of this latter infected cell is shown. × 1,000.

FIG. 3. Film made from placental exudate, Cow 298, aborted. Alkaline methylene blue. The epithelial cell is enlarged, vacuolated, and filled with bacilli. × 1,000.

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[11] Koch, R., Untersuchungen über die Aetiologie der Wundinfectionskrankheiten, Leipsic, 1878.

[12] Mallory, F. B., and Hornor, A. A., *J. Med. Research*, 1912–13, xxvii, 115.

[13] Smith, T., *J. Med. Research*, 1913–14, xxix, 291.

PLATE 21.

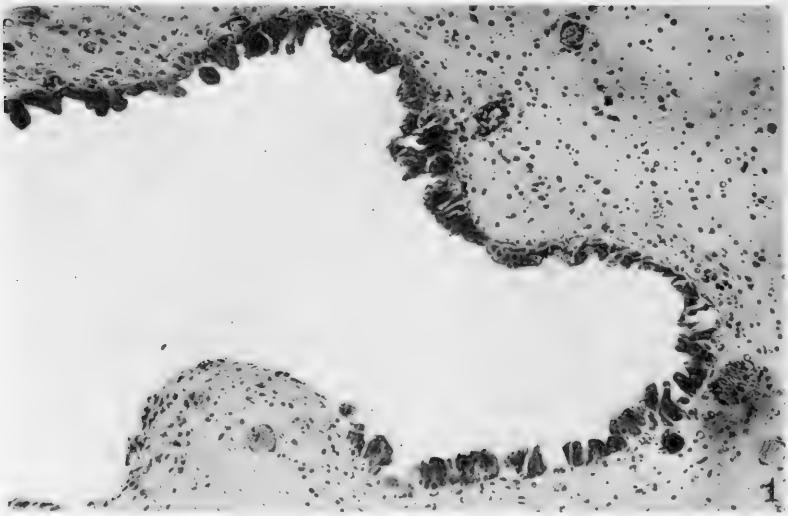
FIG. 4. Placenta, Cow 171. Fetus obtained *in utero* after slaughter. The chorionic epithelium is shown *in situ*. One densely infected cell in the center, others, right and left. The cell nuclei are no longer of normal appearance but the cells themselves are only slightly swollen. × 1,000.

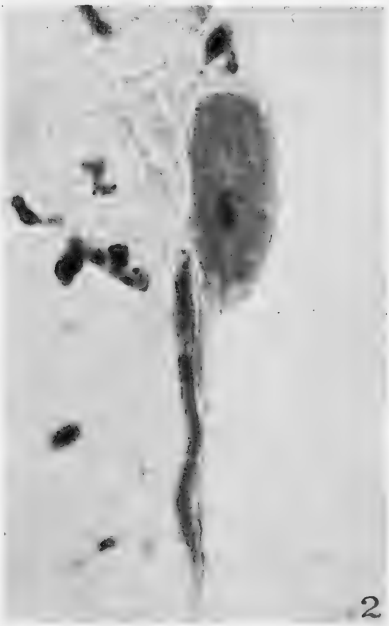
FIG. 5. Placenta, Cow 91, aborted. A short blunt fetal villus from the margin of a cotyledon is shown with all of the covering epithelium filled with bacilli. × 1,000.

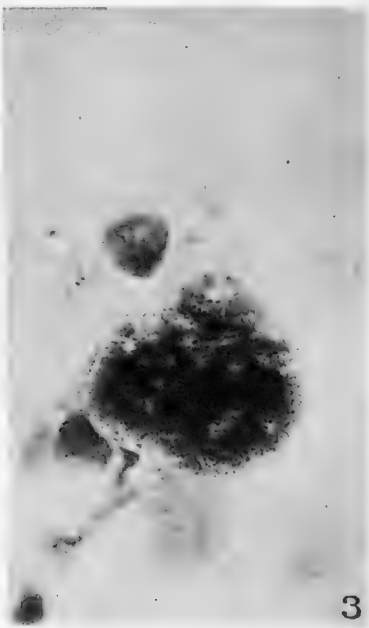
PLATE 22.

FIG. 6. Placenta, Cow 127, which gave birth to a full term calf. The epithelial cells of the chorion are slightly lifted away from their normal base. The cells are much larger than normal, the nuclei pyknotic. All are densely packed with bacilli. × 1,000.

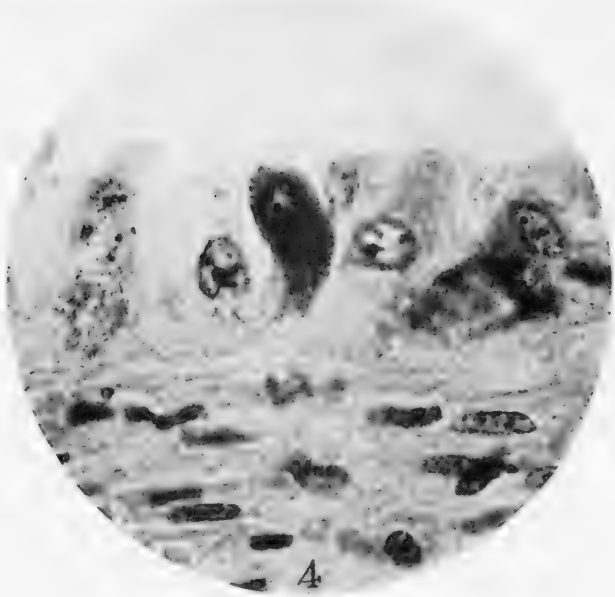
Guinea pigs inoculated with placental tissue of all the above cases, ground up and suspended in salt solution, became diseased and yielded pure cultures of *B. abortus*. In several cases cultures were obtained directly in spite of the soiled condition of placenta.

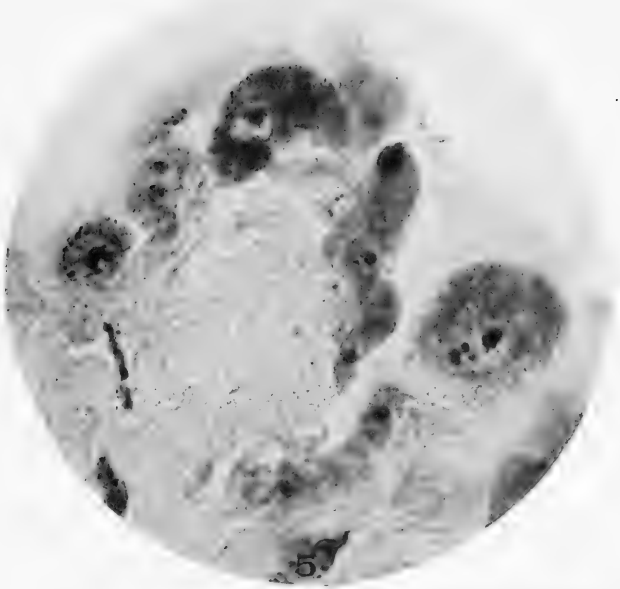






(Smith: Localization of *Bacillus abortus*.)





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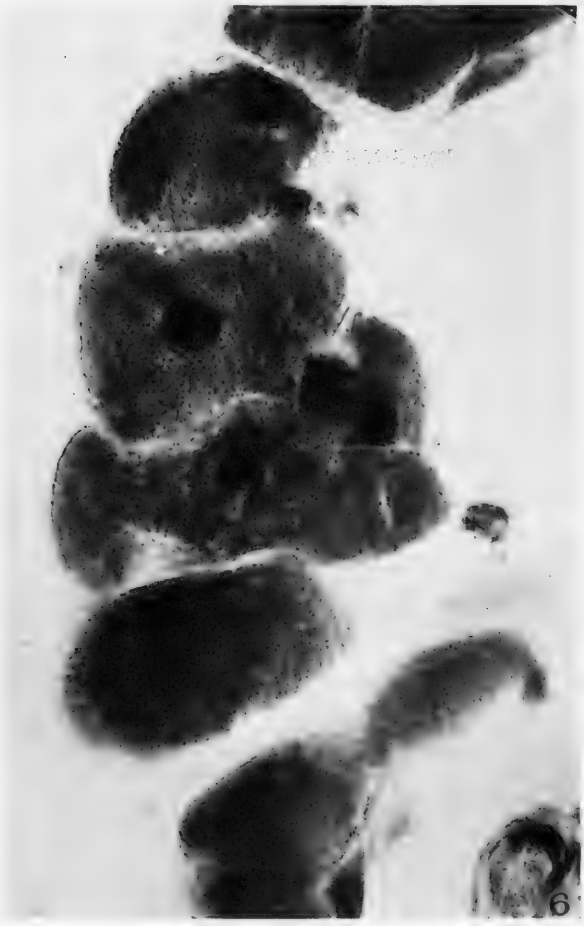

(Smith: Localization of *Bacillus abortus*.)

RESULTS OF PROPHYLACTIC VACCINATION AGAINST PNEUMONIA AT CAMP WHEELER.*

By RUSSELL L. CECIL, M.D.,
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(Received for publication, February 15, 1919.)

INTRODUCTION.

The recently published work of Cecil and Austin (1) on the results of prophylactic inoculation against pneumonia at Camp Upton has furnished considerable evidence that vaccination against Type I, II, and III pneumococci is practical and that it affords satisfactory protection against the pneumonia produced by these types. In view of the widespread prevalence of pneumonia in the American Army, especially in the cantonments, it was obvious that immediate effort should be made toward instituting a more extensive trial of pneumococcus vaccine and, if the results justified it, the establishment of pneumonia vaccination on an efficient and permanent basis.

For the sake of convenience, it might be well to refer briefly to the results obtained by Cecil and Austin at Camp Upton.

From a study of the agglutinins and protective power of the serum of 42 persons vaccinated against pneumococcus, Types I, II, and III, it was demonstrated that a definite immune response could be secured to Types I and II by the dose of vaccine employed. Little evidence of response to Type III could be demonstrated by these methods, but this was of less significance in view of the fact that in animals it is relatively difficult to secure antibodies against this strain even though a considerable degree of active immunity may have been produced in the vaccinated animal. The degree of response to the vaccine appeared to be dependent upon the total dose of each type of pneumococcus ad-

*An official report submitted to the Surgeon General by the Commission appointed to investigate pneumococcus vaccination.

ministered. The greater the dose the greater was the protection elicited, and this remained true whether the vaccine was administered in a single large dose, or in a number of successive small doses.' 12,519 men were vaccinated at Camp Upton (40 per cent of the camp strength) most of the men receiving three or four inoculations at intervals of 5 to 7 days. The total dosage was 6 to 9 billion of Types I and II and 4½ to 6 billion of Type III. The local and general reactions were usually mild, but there were quite a few small sterile fluctuating infiltrations at the site of the injection which appeared to be an expression of cutaneous hypersusceptibility. The results of the vaccination were highly satisfactory. The men were under observation for 10 weeks following vaccination, and, during that time, no cases of pneumonia of the three fixed types occurred among the men who had received two or more injections of vaccine. In a control of approximately 20,000 men, there were 26 cases of pneumonia of Types I, II, and III during the same period. The incidence of Pneumococcus Type IV pneumonia and streptococcus pneumonia was much less in the vaccinated troops than among the unvaccinated. The final figures showed only 17 cases of pneumonia of all types occurring among the 12,519 men who received vaccine, whereas, among the unvaccinated troops, during the same period, there was a total of 173 cases of pneumonia of all types. For the 10 weeks during which the men were under observation, the annual pneumonia death rate for vaccinated troops was only 0.83 per 1,000; for the unvaccinated troops it was 12.8.

In spite of the successful results obtained at Camp Upton, there were certain objections to pneumococcus vaccine, which interfered somewhat with its extensive application. In the first place, three injections were necessary in order to obtain a satisfactory protection. This, however, placed a great burden on regimental surgeons, and was distasteful to the men themselves as they had already received the triple typhoid injections and the smallpox vaccination. Another objection to pneumococcus vaccination was the occurrence of the small sterile infiltrations which sometimes followed its use. These infiltrations were never serious, but caused some anxiety and discomfort to the patient. It was a fortunate coincidence, therefore, that just at this time Colonel Eugene R. Whitmore (2), of the Medical Corps, U. S. Army, made his first report on the use of vegetable oil as a vehicle for the suspension of bacterial vaccines. Some work had already been done along this line in France by Le Moignic and Pinoy (3) who had tried suspending triple typhoid vaccine in vegetable oils, and had met with promising results. Achard and Foix (4) also reported favorably on the use of olive oil as a medium for

suspending bacteria. Whitmore applied this principle to a wide variety of vaccines, including the pneumococcus. These vaccines differed in no essential respect from the saline vaccine, except that the dried bacteria were suspended in olive oil instead of in salt solution. More recently Colonel Whitmore has substituted cottonseed oil containing 2 per cent lanolin for olive oil, as it appears to be less irritating to the subcutaneous tissue. Whitmore, Fennel, and Petersen (2) found that the triple typhoid vaccine in oil produced even better agglutination response in the serum than the same vaccine in saline solution. The reaction, both local and general, following the injection of the typhoid lipovaccine was so mild that the three original doses of saline vaccine could be combined into one dose (1 cc.) of the lipovaccine, without producing any unpleasant results. This mild reaction was probably due to the fact that the oily suspension was more slowly absorbed than the saline suspension. The reduction of typhoid vaccination to one injection presented such an obvious advantage over the old method that the Surgeon General shortly afterwards directed that the lipovaccine be substituted for the saline vaccine altogether, and it is now being universally employed throughout the Army.

Following the work with typhoid lipovaccine, experiments were undertaken at the Army Medical School with pneumococcus lipovaccine, and here again, it was found that pneumococcus vaccine in oil produced good immune reactions, and that large doses could be administered without ill effect. It was therefore decided to prepare, in addition to the typhoid lipovaccine, a pneumococcus lipovaccine of Types I, II, and III, and to supply it to the Army for vaccination of volunteers. Some preliminary experiments carried out by us at Camp Wheeler had shown that 10 billion pneumococci of each of the three types could be injected without producing severe reactions.

About September 1, 1918, the writers of this report were appointed by the Surgeon General as a special commission to investigate the value of pneumonia vaccine, and were directed to proceed to Camp Wheeler, Georgia, for the purpose of instituting voluntary vaccination against pneumonia among the troops. There were good reasons for the selection of Camp Wheeler for this experiment. During the 6 months from October 5, 1917, to March 29, 1918, there had been 917

TABLE I.

Comparison of the Cases of Pneumonia from July 1 to October 1 (Summer Epidemic), with Those from October 1 to December 20, 1918 (Influenza Epidemic).

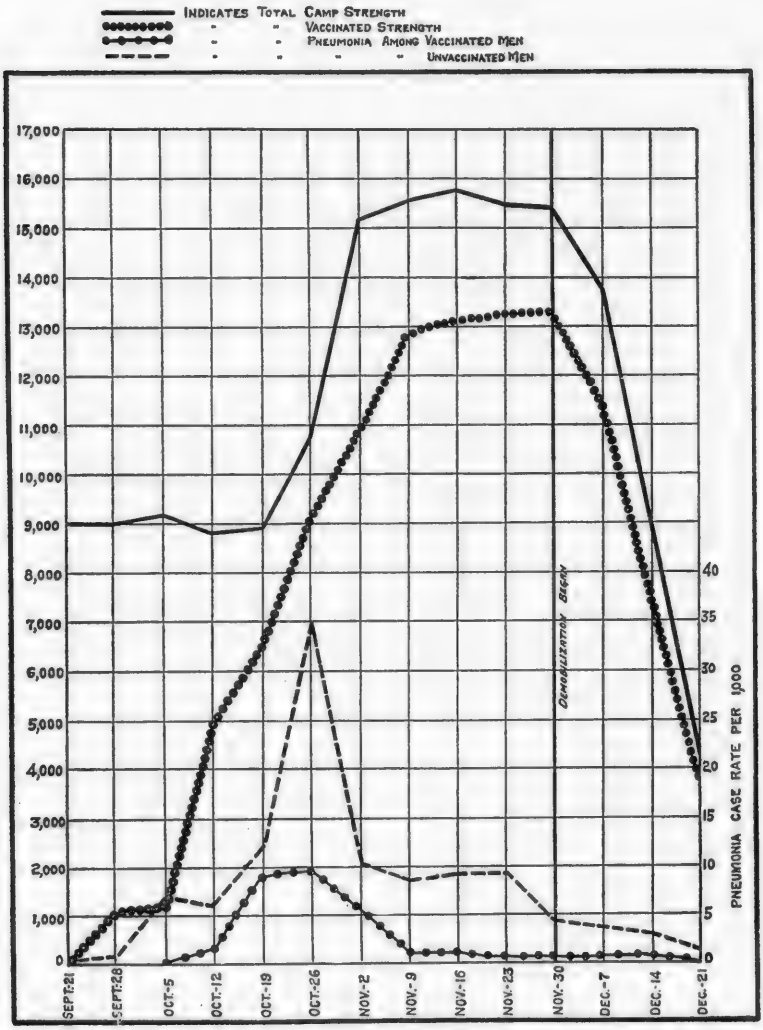
Types.	July 1 to Oct. 1, 1918.				Oct. 1 to Dec. 20, 1918.			
	No. of cases.		No. of deaths.	Mortality.	No. of cases.		No. of deaths.	Mortality.
				per cent				*per cent*
Pneumococcus, I	41		10	24.3	13		4	30.7
" II	21	73 (43.2%)	6	18 28.6	24.6 18	70 (11.5%)	7	23 38.9
" III	11		2	18.2	39		12	30.7
" IV	96	(56.8%)	12	12.5	536	(88.5%)	117	21.6
Hemolytic streptococcus	1		0	0	10		5	50.0
Non-hemolytic "	12		1	8.3	34		2	5.8
Friedländer's bacillus	0		0	0	1		1	100
Undetermined	34		3	8.9	15		2	13.3
Total	216		34	15.7	666		150	22.4

cases of pneumonia at Camp Wheeler, a figure which gave that camp the next highest pneumonia rate of all the camps for that period. Furthermore, in August, 1918, a second epidemic of pneumonia broke out at Camp Wheeler among the negro draft and was the immediate cause of our being sent to that camp.

In Table I it will be seen that for the 3 months, July 1 to October 1, 1918, though it was the hot season, there were 216 cases of pneumonia at Camp Wheeler with 34 deaths. This was nearly all primary pneumonia of the lobar type, and practically all of it of pneumococcus origin. Of the pneumococcus cases, 43.2 per cent were due to the fixed types and 56.8 per cent to Type IV. Nearly half of the pneumonia, therefore, was being caused by the types of pneumococcus against which we proposed to vaccinate.

It soon became apparent, however, that conditions at Camp Wheeler were very different from those which had prevailed at Camp Upton the preceding winter. In the first place, many of the men whom we were to vaccinate at Camp Wheeler were raw recruits of rural origin, a considerable part of them negroes. The men vaccinated at Camp Upton had come chiefly from New York City, were well seasoned at the time of vaccination, and were mostly of the white race. In the second place, the situation at Camp Wheeler was complicated by the influenza epidemic, which, of course, had been absent at Camp Upton.

By referring to Text-fig. 1, it will be seen that on September 21, 1918, the date on which vaccination of troops at Camp Wheeler was initiated, the total strength of the camp was about 9,000 men. The population remained practically unchanged until October 16 when a draft of 1,270 negroes arrived. During the following 2 weeks a large draft of white troops arrived, some from New York State and others from Alabama. By November 2, the population of the camp had increased from 9,000 to something over 15,000, and by November 16, the maximum population, nearly 16,000, was reached. On November 30, demobilization began, and during the next 3 weeks the population was reduced from 15,000 to 4,000 men. The troops who constituted the camp in September and the earlier part of October were practically all seasoned men. We succeeded, as shown by the chart, in getting 75 per cent of the old troops vaccinated before the draft arrived.



TEXT-FIG. 1. Pneumonia at Camp Wheeler from September 21 to December 21, 1918.

The new draft began on October 16 and was completed by November 2. The incoming men were examined by a medical officer as they got out of the train. Any men who were sick were sent at once to the Base Hospital. All the others were marched directly to the vaccinating pavilion, and received at once their pneumococcus vaccine. In this way most of the recruits were vaccinated against pneumonia within 24 hours after arriving at camp. All those who objected to taking the vaccine were passed by.

Technique of Vaccination.

The men were arranged in single file and instructed to roll up the left sleeve. As the troops filed into the vaccinating building, a record of each man's name, age, race, organization, rank, nativity, and duration in service was taken on one of the so called "Sick and wounded cards" (Form 52). On the lower part of the card was stamped the date and dosage of vaccination. After giving the necessary information, the men passed down a narrow aisle and were vaccinated as ollows: An enlisted man swabbed the posterior aspect of the left arm half way between the elbow and shoulder with tincture of iodine. A medical officer then injected 1 cc. of pneumococcus vaccine subcutaneously, using a needle of fairly small caliber. Then, in order to prevent the escape of any of the oily suspension, another enlisted man pressed a piece of absorbent cotton against the point of puncture and instructed the man to hold it there for a minute or two.

In addition to the pneumococcus vaccine 2,226 recruits received an injection of influenza vaccine (1 billion influenza bacilli) in the right arm. The results of the vaccination against influenza will be considered in a separate report. The reaction produced by influenza vaccine was almost always very mild. After the men had received the vaccine, they were marched to their quarters and given complete rest for 24 hours.

By referring again to Text-fig. 1, it will be observed that the recruits were vaccinated almost as rapidly as they came to camp and that by November 9, 13,000 men in all had received pneumococcus vaccine, about 80 per cent of the camp strength. In other words, from October 19 to December 20, a period of 2 months, 75 to 80 per

cent of the entire population of the camp consisted of men who had been vaccinated against pneumonia.

Table II indicates the number of men vaccinated and classified according to whether they were white or negro, recruits or seasoned men. We arbitrarily defined the term "recruit" to mean any man who had been in the service 1 month or less. Table II shows that altogether 3,832 white recruits (80 per cent of the total number) and 1,230 negro recruits (86 per cent of the total number) received the pneumococcus vaccine, making a grand total of 5,062 recruits who were inoculated. Of the seasoned men, 6,687 white troops (75 per

TABLE II.

Data on Pneumococcus Vaccination.

Race.	No. vaccinated.			Approximate No. unvaccinated.		
	Recruits.*	Seasoned men.	Total.	Recruits.	Seasoned men.	Total.
White	3,832	6,687	10,519	919	2,156	3,075
Negro	1,230	1,711	2,941	194	146	340
Total	5,062	8,398	13,460 (80% of camp strength).	1,113	2,302	3,415 (20% of camp strength).

* All men in service for 1 month or less were classified as recruits.

cent) and 1,711 negro troops (92 per cent), a total of 8,398, were inoculated against pneumonia. Altogether, 10,519 white men and 2,941 negroes were vaccinated with the pneumococcus lipovaccine, making a grand total of 13,460 men, or 80 per cent of the entire camp strength.

For the purposes of control, it would have been more desirable to have vaccinated only half of the camp; that is, only half of each organization. As this method, however, was not feasible, and in consideration of the serious influenza epidemic, it was decided to vaccinate as large a percentage of the camp as possible with the hope of reducing the pneumonia rate to a minimum.

Character of Reactions to Pneumococcus Lipovaccine.

Local Reaction.—Pneumococcus lipovaccine produces a mild local reaction, which is distinctly less marked than that caused by triple typhoid lipovaccine, though the former contains more than three times as many bacteria as the latter. That the lipovaccine is much less irritating than the saline vaccine is evidenced by the fact that while the saline preparation often produces a sterile fluctuating infiltration at the site of injection, only five such reactions (Table III) were observed among the 13,000 men vaccinated with lipovaccine at Camp Wheeler. This figure is in strong contrast to the 152 infiltrations encountered in the Camp Upton experiment. This type of local reaction is never serious, but causes some concern and discomfort to the patient, and its frequent occurrence would certainly militate against the general use of pneumococcus vaccine. In our opinion, the elimination of these infiltrations marks one of the most important advances in prophylactic pneumococcus vaccination.

Doses of pneumococcus lipovaccine three times as large as the dose finally decided upon were administered to volunteers at Camp Wheeler without any serious local or general reaction. It is almost incredible that such huge numbers of pathogenic bacteria (90 billion) can be injected beneath the skin with so little untoward effect.

Constitutional Reaction.—Like the local reaction, the constitutional reaction to pneumococcus lipovaccine is usually insignificant. In many cases it is entirely absent. Of the 13,460 men who received the vaccine at Camp Wheeler, only 104 (0.7 per cent) were sufficiently affected to be admitted to the Base Hospital (Table III). It is especially interesting to note that while 5,062 recruits and 8,398 seasoned men were inoculated, 101 of the admissions to the hospital were recruits, and only 3 were seasoned men, a fact which would indicate that practically 100 per cent of men in good physical condition can take the vaccine without any particular discomfort.

It is also important to note that 80 of the 104 admissions were among the 2,226 recruits (44 per cent) who received the pneumococcus and influenza vaccine simultaneously; only 24 occurred among the 2,836 recruits (56 per cent) who received pneumococcus vaccine alone.

The negroes seemed more susceptible to the pneumococcus toxin than whites; 65 per cent of the hospital admissions were negroes,

TABLE III.

Hospital Admissions for Pneumococcus Vaccine Reactions.

Severe local reactions			5 cases.
General reactions.	Recruits.	Seasoned men.	Total.
White	34	2	36
Negro	67	1	68
Total	101	3	104
Day of admission.			
Day of vaccination			9 cases.
" after "			48 "
2 days after "			23 "
3 " " "			13 "
4 " " "			5 "
5 " " "			6 "
Total			104 "
Symptoms.			
Headache			88 cases (84 per cent).
Backache			37 " (35 " ").
General muscular aching			39 " (38 " ").
Malaise			22 " (21 " ").
Chill			21 " (20 " ").
Pain in chest			18 " (17 " ").
Cough			13 " (12 " ").
Sore throat			11 " (10 " ").
Nausea			2 "
Anorexia			1 "
Nosebleed			1 "
Length of time in hospital.			
1 day			7 cases.
2 days			30 "
3 "			22 "
4 "			13 "
5 "			9 "
More than 5 days			23 "
Total			104 "
Fever cases.			
Temperature. °F.			
98– 99			12 cases.
99–100			14 "
100–101			15 "
101–102			23 "
102–103			17 "
103+			23 "
Total			104 "
History of pneumonia			13 cases.

though they constituted only 21.8 per cent of the total number of men vaccinated. Only 13 of the hospital admissions gave a previous history of pneumonia.

Most of the men with sharp reactions reported to the hospital within 48 hours after receiving the inoculation, but there were a small number who did not appear until the 4th or even the 5th day after injection. Apparently the slower absorption of the oily emulsion sometimes causes a delayed reaction. These men were in no instance seriously ill, though some of them were quite uncomfortable.

The symptom most often complained of was headache. Backache and general muscular aching were also very common. The symptoms described were headache (84 per cent), backache (35 per cent), general muscular aching (38 per cent), general malaise (21 per cent), chill (20 per cent), pain in chest (18 per cent), cough (12 per cent). A rise in temperature was noted in 89 per cent of the hospital admissions, but this was usually not marked. In 64 cases the temperature never rose above 102° F., and in most cases it was normal 24 or 48 hours after admission. As a rule, these patients remained in the hospital only 2 or 3 days. They rarely stayed longer than 5 days.

Method of Keeping Records.

In order to keep a careful record of all cases of pneumonia that developed in camp subsequent to vaccination, the vaccination register cards were filed (1) according to organization; (2) carbon copies were filed alphabetically; (3) a special file was prepared from the soldiers' identification numbers. Whenever a case of pneumonia was reported, a search was made for the patient's name in all three files. In addition to searching the file, the patient himself was carefully questioned as to whether or not he had received the pneumonia vaccine. By this method of procedure, the percentage of error in our results was probably reduced to a very low figure.

Sputum Examination.

Realizing from the outset the great importance of accurate bacteriological study on all cases of pneumonia, we made arrangements for a large supply of white mice and were able to maintain this supply

throughout the entire experiment. In this way practically every sputum examined was tested by the mouse method. In a series of 690 cases of pneumonia, there were only 17 cases in which the sputum was not examined. It had been our original intention to check every sputum, either by examination of a second specimen or by having two independent observers make examinations on the same specimen. During the height of the influenza epidemic, however, it was found impossible to maintain this method of control on every case. Of the 673 cases of pneumonia in which a sputum analysis was made, 368 were controlled by a second examination or by a second observer. The second examination was not always made on a specimen of sputum, however. In some instances it was a positive blood culture, an empyema exudate, or cultures taken directly from the lung at autopsy. In the majority of instances, the two examinations were consistent, and often where the findings were inconsistent, there was good reason to believe that we were dealing with a mixed infection. In the final classification of pneumonia cases, however, we have tried to avoid a group of mixed infections and, in some cases where two or more organisms have been found, have classified them according to the virulence of the types. For example, in a number of cases, Type IV pneumococcus was found in association with one of the fixed types of pneumococcus, and such cases were always classified as fixed types.

Results of Vaccination.

The results of vaccination against pneumonia, as shown by the incidence of pneumonia in vaccinated and unvaccinated troops, are indicated in Tables I, IV, and VI. In Table I the classification of all cases of pneumonia occurring at Camp Wheeler between July 1 and October 1, 1918, is compared with a classification of all the cases of pneumonia occurring at this camp from October 1 to December 20, 1918. From these figures it will be observed (1) that the incidence of pneumonia for the 3 months July 1 to October 1 was comparatively high, in view of the season of the year; (2) that there were three times as many cases of pneumonia during the following 3 months (October 1 to December 20) due to the influenza epidemic; (3) that pneumonia

TABLE IV.

Cases of Pneumonia at Camp Wheeler from September 21 to December 20, 1918 (Period of Experiment). Number of Men Vaccinated against Pneumonia, 13,460 (about 90 Per Cent of Strength of Camp).

Types.	Vaccinated.						Unvaccinated.					
	No. of cases.			No. of deaths.		Mortality.	No. of cases.			No. of deaths.		Mortality.
						per cent						*per cent*
Pneumococcus, I	7	32	(9.6%)	2	10	(13.6%) 28.5	6	42	(14%)	2	14	(25.5%) 33.3
" II	3*			1		33.3 31.2	16†			6		37.5 33.3
" III	22			7		31.8	20			6		30.0
" IV	298		(90.4%)	63		(86.4%) 21.1	256		(86%)	55		(74.5%) 21.4
Hemolytic streptococcus	5			3		60.0	5			2		40.0
Non-hemolytic "	21			2		9.5	13			0		0
Friedländer's bacillus	0			0		0	1			1		100
Undetermined	7			1		14.2	10			1		10.0
Total	363			79		21.7	327			73		22.3

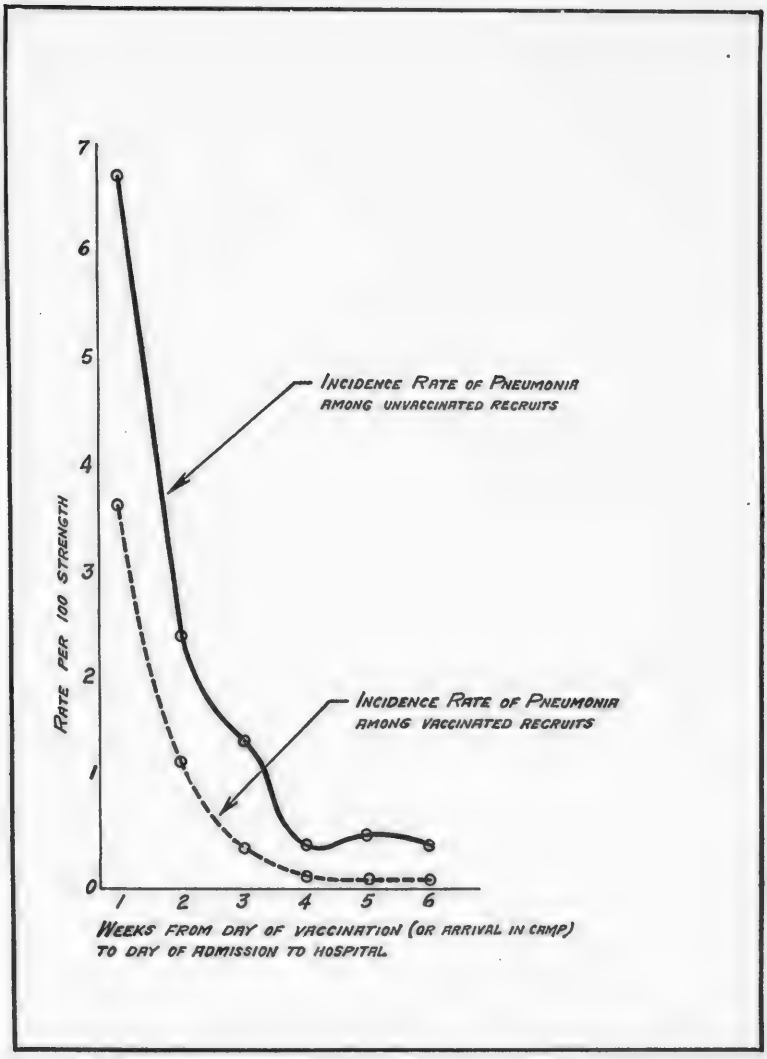
* These three cases were all atypical Type II.

† Of these sixteen cases ten were typical Type II, and six were atypical Type II.

of Types I, II, and III constituted 43.2 per cent of all the pneumococcus cases in the period from July 1 to October 1, while in the period from October 1 to December 20, Type I, II, and III pneumococci caused only 11.5 per cent of all the pneumococcus pneumonia; (4) that the mortality rate for all cases was 15.7 per cent during the summer months and 22.4 per cent during the influenza epidemic.

Table IV shows the relative incidence of pneumonia among vaccinated and unvaccinated troops during the period of the experiment, September 21 to December 20, 1918. In comparing these figures it should be borne in mind that during most of this period 80 per cent of the men were vaccinated against pneumonia. Examination of Table IV shows that, during these 3 months, there were 32 cases of pneumonia of Types I, II, and III among vaccinated men and 42 cases of pneumonia of these types among the unvaccinated; there were 298 Type IV pneumonia cases among the vaccinated men, as compared with 256 Type IV cases among the unvaccinated. In other words, 9.6 per cent of the pneumococcus cases were caused by fixed types of pneumococcus in the vaccinated group and 14 per cent were caused by the same types among the unvaccinated. Altogether, there were 363 cases of pneumonia during this period among vaccinated men and 327 among the unvaccinated. The incidence of pneumonia was about the same in the two groups, though the vaccinated group represented 80 per cent of the camp strength. The mortality rate in the vaccinated series, 21.7 per cent, was slightly lower than that for the unvaccinated series, 22.3 per cent.

These figures are not altogether encouraging, but a more careful analysis shows that other factors have to be considered before any just estimate of the value of the vaccine can be arrived at. In the first place, Text-fig. 1 shows clearly that the weekly incidence rate of pneumonia was conspicuously lower among the vaccinated than among the unvaccinated troops. It will be observed that the scope of the two rate curves is about the same between October 12 and 19, the time at which the influenza epidemic began to make itself felt at Camp Wheeler. The following week, as the vaccinated men began to develop protection, the rate for the vaccinated ceased to increase, then rapidly declined. Had these men remained unvaccinated, the pneumonia rate for this particular group would almost certainly have



TEXT-FIG. 2. Comparison of the pneumonia case rate (all types) among vaccinated and unvaccinated recruits.

reached a high peak by October 26 as did that for the unvaccinated group.

Text-fig. 2 shows that a large percentage of pneumonia cases occurring among the troops at Camp Wheeler developed among the recruits, whether vaccinated or unvaccinated, during the first 2 weeks of their residence in camp.

Colonel Whitmore in his recent Harvey lecture (5) reports some experiments with pneumococcus lipovaccine which show that immunity

TABLE V.

Showing Development of Protective Bodies against Pneumococcus Type I in Human Serum Following Injection of 1 Cc. of Pneumococcus Lipo-Vaccine.

Date.	Control.		Nov. 17	Nov. 18	Nov. 19	Nov. 20	Nov. 21	Nov. 22	Nov. 23	Nov. 25	Nov. 26	Nov. 28	Nov. 29	Dec. 1	Dec. 4	Dec. 20
Days after vaccination.			Before.		1	2	3	4	5	7	8	10	11	13	16	32
0.000001 cc	30	30	30	36	36	36	30	24	24	36	S.	S.	S.	S.	S.	S.
0.00001 cc	30	36	30	24	30	24	30	30	30	36	48	"	"	"	"	"
0.0001 cc	30	X.	22	24	22	24	24	30	30*	30	30	30	30	30	"	24
0.001 cc	30	"	22	22	22*	24*	15	15	X.	24	36	30	22	30	22	30
0.01 cc	22	"	X.	22	X.	X.	22*	15	"	X.	10	30*	15	24	22	15
0.1 cc	X.	"	"	X.	"	"	X.	15	"	"	X.	X.	15	15	22	15

Private G. vaccinated November 18, 1918, 4 p.m. Vaccine 2 mg. per cc.

Protection test December 21, 1918. 0.2 cc. of serum + multiple of minimum lethal dose of Pneumococcus Type I broth culture injected intraperitoneally into mice. Time of death noted in hours. Pneumococci found in all peritoneal exudates.

X. indicates no test; S. survival; * 0.1 cc. of serum.

Vaccine contained 0.83 mg. (about 10 billion) of each of the three fixed types, I, II, and III, pneumococcus.

against the pneumococcus does not begin to develop until the 8th day after injection of the vaccine. From that time on the immunity curve rises steadily and continues to rise for some time thereafter. Table V shows the results obtained in one of Colonel Whitmore's experiments.[1] The serum was tested before vaccination and found to have no protective power value for mice. The individual was vaccinated on November 18, and his blood taken on successive days

[1] Colonel Whitmore has kindly permitted us to publish this table.

for the purpose of studying the development of protective bodies. It will be seen from the table that there were no survivals among the mice until the 8th day after vaccination, and that from that time on the percentage of survivals increased from day to day.

With this consideration in mind, we have prepared a revised table of pneumonia cases occurring among the vaccinated men (Table VI). In this table all cases of pneumonia developing within 1 week after vaccination have been excluded. When this alteration is made, there remain only two cases of Type I pneumonia, one case of Type II (and that an atypical Type II), and five cases of Type III develop-

TABLE VI.

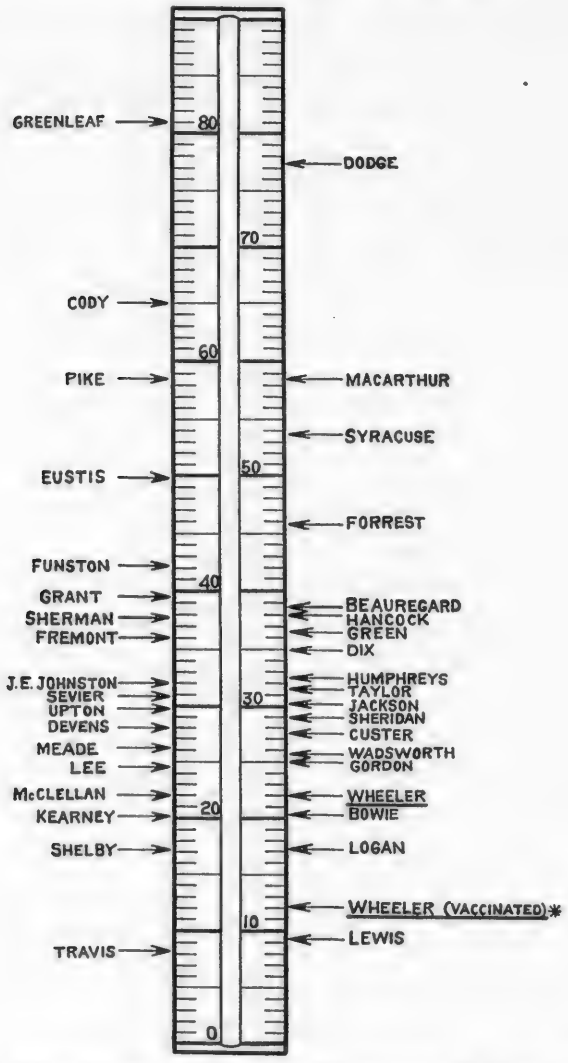
*Revised Table of Incidence of Pneumonia among Vaccinated Men from September 21 to December 20, 1918.**

Types.	No. of cases.		No. of deaths.		Mortality.
					per cent
Pneumococcus, I.....	2		0		0
" II.....	1† } 8	(5.8%)	0 } 1	(6.7%)	0 } 12.5
" III.....	5		1		20
" IV.....	124	(94.2%)	14	(93.3%)	11.2
Hemolytic streptococcus.....	4		2		50.0
Non-hemolytic "	14		1		14.2
Undetermined.....	5		1		20.0
Total.....	155		19		12.2

* In this table all cases of pneumonia developing within 1 week after inoculation have been excluded.

† This case was an atypical Type II.

ing among vaccinated men during the period of the experiment. That there were five Type III cases in this group is not so surprising when we recall the difficulty one has in immunizing animals against Type III pneumococcus. The most important and significant point, however, in connection with these eight cases of pneumonia is the fact that all eight cases followed severe attacks of influenza. Only one of the eight cases died, and this was one of the Type III group. If all pneumonia patients developing within a week after vaccination are excluded from the Type IV group of vaccinated pneumonia cases, the figure is reduced from 208 to 124 cases. 103 of the 124 cases were



TEXT-FIG. 3. Mortality rate per 100 cases of pneumonia in Army camps from September 21 to December 20, 1918.

* Rate for 155 cases of pneumonia that developed among vaccinated men 1 week or more after vaccination.

instances of secondary pneumonia and only 21 primary. There were 14 deaths among the 103 secondary pneumonia cases and no deaths in the series of 21 primary pneumonia cases.

The mortality rate for the 155 cases of pneumonia of all types that developed 1 week or later following vaccination was only 12.2 per cent. When this figure is compared with the pneumonia mortality rate for the various camps during the same period (Text-fig. 3), it will be seen that the rate for vaccinated pneumonias at Camp Wheeler was one of the lowest of any of the camps. The mortality rates for other camps were calculated from the Surgeon General's "Weekly sick report," and are accurate only in as far as the figures on incidence of pneumonia and deaths from pneumonia in the respective camps are accurate.

Pneumonia among Recruits.

The influenza epidemic made its first appearance at Camp Wheeler on September 30 when about 40 cases were admitted to the hospital from the Camp Stockade for General Prisoners. These men, the first to import the infection, were negro draft evaders from rural parts of Georgia who had failed to report at the time of the last draft, September 1, and who had since been rounded up as general prisoners. As soon as these draft evaders, who numbered about 150, had been mustered into service, those not in the hospital with influenza were transferred gradually to the negro companies of the Receiving Camp. Influenza was transferred to this organization with them and reached a maximum daily admission rate of 69 cases on October 4.

On October 16, 17, and 18, 1,270 negro recruits arrived in camp from rural sections of Georgia. 1,113 of these men were vaccinated for pneumonia within 24 hours after arrival. Between October 23 and 27, 4,751 white recruits arrived, approximately 3,500 from western New York State, the remainder from rural sections of Alabama. 3,382 of these men were immediately vaccinated.

During their 1st week in camp 419 negro recruits, 33 per cent of their command, developed influenza. Of these, 72 cases, or 17.2 per cent, were followed by pneumonia. These men arrived at Camp Wheeler already exposed to or infected with influenza, and were an

especially susceptible group since the epidemic had not at that time generally reached the rural counties of Georgia. 533 of the white recruits, 11.2 per cent of their strength, developed influenza during their 1st week in camp, followed by 89 cases of pneumonia, a rate of 16.7 per cent. It therefore appears that this particular group of negroes was much more susceptible to influenza than the whites; but when they had once become infected, the chances of developing pneumonia were about the same. The white recruits from New York State came from a section already generally infected with influenza.

Text-fig. 2 aims to show the relative incidence (by week) of pneumonia among vaccinated and unvaccinated recruits immediately following their arrival in camp. The bacteriological types have been left out of consideration, Pneumococcus Type IV and other organisms being included with the three types which compose the vaccine. The curves express a rate which in either case is very high for the 1st week, due to the fact that practically all of the group of recruits arrived in camp already exposed to or infected with influenza. 6.7 per cent of the unvaccinated and 3.6 per cent of the vaccinated recruits developed pneumonia within 1 week after arrival in camp. The rates for the 1st week would doubtless have been more nearly the same had it not been for the fact that those already infected with influenza were not vaccinated. As immunity does not develop until after the 8th day, it is reasonable to expect the incidence among the vaccinated to remain high during the 1st week and even during the 2nd week, as the latter group was being infected with influenza at the same time that the lipovaccine was building up resistance against pneumonia.

It is noteworthy that the weekly pneumonia rate among the vaccinated recruits remained consistently only about one-half of that for the unvaccinated. 72 unvaccinated recruits developed pneumonia during the week following vaccination, 6.7 per cent of their strength; 180 vaccinated recruits, 3.6 per cent of their strength. 53 unvaccinated recruits developed pneumonia after the 1st week, 4.9 per cent of their strength; 108 vaccinated recruits, 2.2 per cent of their strength. Furthermore, there is certainly no evidence to show that the administration of pneumococcus vaccine has in any way predisposed to an attack of pneumonia. Had such been the case the inci-

dence rate for the 1st week among the vaccinated would doubtless have exceeded that for the unvaccinated. As it was, the former was considerably inferior to the latter.

Pneumonia among Recruits Compared with Pneumonia among Seasoned Men.

Table VII shows that in recruits pneumonia secondary to influenza has proved more fatal than primary pneumonia, the death rate being 25.3 per cent for the former, 13.8 per cent for the latter. Moreover,

TABLE VII.

Mortality Rates for a Series of 412 Cases of Pneumonia among Recruits. Primary Pneumonia and Pneumonia Secondary to Influenza.

Classification of recruits.		Primary.			Secondary.		
		No. of cases.	No. of deaths.	Mortality.	No. of cases.	No. of deaths.	Mortality.
				per cent			*per cent*
White.	Vaccinated	6	1	16.7	142	36	25.3
	Unvaccinated	1	0	0.0	58	15	25.8
Negro.	Vaccinated	17	1	5.9	123	32	26.0
	Unvaccinated	5	2	40.0	60	14	23.3
Total.	Vaccinated	23	2	11.5	265	68	25.7
	Unvaccinated	6	2	33.3	118	29	24.6
Grand total		29	4	13.8	383	97	25.3

in primary pneumonia it will be observed that the death rate for vaccinated cases is only one-third that for unvaccinated cases, 11.5 and 33.3 per cent respectively; whereas in secondary pneumonia the death rate is practically the same in the vaccinated and unvaccinated groups.

The seasoned men (Table VIII) show a somewhat higher death rate for pneumonia secondary to influenza than for primary pneumonia. Again, however, a marked contrast is observed in the mortality rate of primary pneumonia in vaccinated and unvaccinated men, the rate in unvaccinated (31.2 per cent) being twice that of the vaccinated (15.8 per cent). And again the death rate in secondary pneumonia

TABLE VIII.

Mortality Rate for a Series of 168 Cases of Pneumonia among Seasoned Troops. Primary Pneumonia and Pneumonia Secondary to Influenza.

Classification of seasoned men.		Primary.			Secondary.		
		No. of cases.	No. of deaths.	Mortality.	No. of cases.	No. of deaths.	Mortality.
				per cent			*per cent*
White.	Vaccinated	5	1	20.0	29	5	17.3
	Unvaccinated	3	1	33.3	74	16	21.6
Negro.	Vaccinated	14	2	14.3	13	1	7.9
	Unvaccinated	13	4	30.7	17	1	5.9
Total.	Vaccinated	19	3	15.8	42	6	14.3
	Unvaccinated	16	5	31.2	91	17	18.7
Grand total		35	8	22.9	133	23	17.3

TABLE IX.

Pneumonia Rate per 1,000 for Recruits and Seasoned Men during the 3 Months of the Investigation.

Classification of men.		Absolute No. of men.	Absolute No. of cases of pneumonia.	Incidence per 1,000 men.
Recruits.				
White.	Vaccinated	3,832	148	38.7
	Unvaccinated	919	59	64.0
Negro.	Vaccinated	1,113	140	125.0
	Unvaccinated	157	65	413.0
Total vaccinated recruits		4,945	288	58.2
" unvaccinated "		1,076	124	115.2
Seasoned men.				
White.	Vaccinated	6,570	34	5.2
	Unvaccinated	1,919	77	40.0
Negro.	Vaccinated	1,828	27	14.8
	Unvaccinated	383	30	78.4
Total vaccinated seasoned men		8,398	61	7.2
" unvaccinated " "		2,302	107	46.4

differs very little for the vaccinated and unvaccinated series. It would appear, therefore, from Tables VII and VIII that the death rate in primary pneumonia is favorably influenced by pneumococcus vaccination, but is slightly, if at all, affected by it in pneumonia secondary to influenza. The series of primary cases, however, in both recruits and seasoned men is too small to allow of definite conclusion.

Table IX is a comparison of the pneumonia rate per 1,000 men in recruits and seasoned men during the period of the experiment. It was hardly possible to determine the annual pneumonia incidence rate for each group, as the various organizations composing a group were vaccinated at different times. Table IX shows: (1) that the pneumonia rate was considerably higher among negro troops than among white troops, and that this difference was quite marked, whether the men were recruits or seasoned men; (2) that the pneumonia rate for any particular group, white or negro, recruits or seasoned men, was markedly lower for the vaccinated part of the group than for the unvaccinated part. This difference was most marked in the case of seasoned white men where the pneumonia rate for unvaccinated men was nearly seven times that for the vaccinated men.

DISCUSSION.

It is necessary to emphasize again the fact that conditions at Camp Wheeler were not nearly so favorable for a test of pneumococcus vaccine as they had been at Camp Upton. The high incidence of pneumonia among recruits at Camp Wheeler shows how much more susceptible they are to pneumonia than seasoned men such as were vaccinated at Camp Upton. In other words, the natural immunity of recruits is much lower than that of seasoned troops, and this is particularly true of rural men, such as composed the Camp Wheeler draft. The Camp Upton division was composed chiefly of New York City men.

A combination of influenza vaccination with pneumococcus vaccination in 2,226 men was another complicating factor at Camp Wheeler, but there is no evidence that the simultaneous injection of the two interfered in any way with the effectiveness of the pneumococcus vaccine.

The influenza epidemic was, of course, the greatest disturbing element in the Camp Wheeler experiment. The type of influenza which has been recently prevalent certainly causes a marked reduction in the patient's resistance to pneumonia. It is possible that a vaccine which, under ordinary circumstances, would have given complete protection against primary lobar pneumonia would fail completely during an epidemic of such virulence as the recent influenza epidemic. Furthermore, it is reasonable to suppose that with the resisting power of the lungs at such a low ebb, even had pneumococcus infection with Types I, II, and III been prevented by means of a vaccine, some other type of pneumonia would have developed in its place. It will be recalled that at the onset of the influenza epidemic at Camp Wheeler, the proportion of Type IV pneumonia cases greatly increased even among the unvaccinated men. This may have been due to the fact that, from that time on, pneumonia at Camp Wheeler ceased almost entirely to be a primary disease, and became a complication of influenza. Under such circumstances infection by whatever virulent organism that happened to be in the mouth would have occurred, and as the Type IV pneumococcus is the type so frequently present in the normal mouth, it would naturally play a prominent part in the etiology of secondary pneumonia.

The epidemic at Camp Wheeler was about over December 1, and the effect of vaccination against pneumonia under more normal conditions could have been observed had not demobilization occurred at this time.

It is apparent that any sort of vaccination against pneumonia must, of necessity, be put to a very crucial test. Typhoid fever, for example, is a primary infection, and the typhoid bacillus rarely finds its way into the alimentary canal of healthy men. The pneumococcus, on the contrary, is frequently found in the healthy mouth and the host is therefore constantly exposed to infection at some moment when his resistance is at a low point. Moreover, pneumonia, in many of the camps at least, has been largely a secondary infection, overtaking a patient when the natural resistance has been lowered to such a degree that pulmonary infection of some kind is almost inevitable. In cases of this kind the problem is to reduce the chances of the patient's becoming infected with a highly virulent organism, so that if pneumonia does develop, the disease will run a mild course.

The present study has not been altogether satisfactory, but it has served to bring out certain points with a fair degree of definiteness. Though 80 per cent of the population at Camp Wheeler were vaccinated, almost as many cases of pneumonia developed among the unvaccinated one-fifth as occurred among the vaccinated four-fifths. If we reckon from 1 week after vaccination, the time when the individual's immunity begins to develop, only 8 cases of Type I, II, and III pneumonia occurred among the vaccinated men, and all these were secondary to severe attacks of influenza. By using the same standard, 124 cases of Type IV pneumonia developed among the vaccinated troops, and 103 of these were also secondary to influenza. Furthermore, it has been demonstrated that the weekly incidence of pneumonia (all types) among both recruits and seasoned men has been more than twice as great among the unvaccinated as among the vaccinated.

These investigations also show that the most striking results of pneumococcus vaccination are obtained with well seasoned men, where the pneumonia incidence rate per 1,000 men was only 7.2 per cent for vaccinated men and 46.4 per cent, almost seven times as great, for unvaccinated men. These findings are in complete accord with those at Camp Upton last winter where only seasoned troops were vaccinated and where the pneumonia incidence rate was ten times as great for unvaccinated men as for vaccinated. We are inclined to believe that the best time to vaccinate recruits would be 2 or 3 weeks before they come to camp.

The large number of cases of Type IV pneumonia in the present epidemic strongly emphasizes the need of a Type IV pneumococcus vaccine. Before an efficacious Type IV vaccine can be obtained, however, much careful study of the biological characteristics of this group will be necessary.

With the cessation of the war, the question naturally arises: How much use can be made of pneumococcus vaccine in civil life? Vital statistics show that pneumonia now kills more people in the United States than any other infectious disease, and the rate seems to be increasing slowly every year. Furthermore, a majority of the pneumonias seen in civilian adults is of the primary lobar type, which is the type of pneumonia most amenable to prophylactic vaccination.

If methods can be devised for still further reducing the toxicity of pneumococcus vaccine, and if active educational propaganda are instituted in its behalf, there is no reason why vaccination against pneumonia should not find a large field of usefulness in civilian as well as in Army life.

SUMMARY.

1. 13,460 men, or about 80 per cent of the entire camp strength, were vaccinated against pneumonia with pneumococcus lipovaccine.

2. The dosage employed in all cases was 1 cc. of the lipovaccine containing approximately 10 billion each of Pneumococcus Types I, II, and III.

3. Both the local and general reactions produced by the vaccine were usually mild. Only 0.7 per cent of those who received the vaccine were sufficiently affected to need hospital care. None of these was seriously ill, and a majority of them returned to duty on the 2nd or 3rd day after admission.

4. Most of the troops inoculated were under observation for 2 or 3 months after vaccination. During this period there were 32 cases of Pneumococcus Type I, II, and III pneumonia among the vaccinated four-fifths of camp, and 42 cases of pneumonia of these types among the unvaccinated one-fifth of camp. If, however, all cases of pneumonia that developed within 1 week after vaccination are excluded from the vaccinated group, there remain only 8 cases of pneumonia produced by fixed types, and these were all secondary to severe attacks of influenza. This exclusion is justified by the fact that protective bodies do not begin to appear in the serum until the 8th day after injection of pneumococcus lipovaccine.

5. There is no evidence whatever that pneumococcus vaccine predisposes the individual even temporarily toward either pneumococcus or streptococcus pneumonia.

6. The weekly incidence rate for pneumonia (all types) among the vaccinated troops was conspicuously lower than that for the unvaccinated troops.

7. The pneumonia incidence rate per 1,000 men during the period of the experiment was twice as high for unvaccinated recruits as for vaccinated recruits, and nearly seven times as high for unvaccinated seasoned men as for vaccinated seasoned men.

8. Influenza causes a marked reduction in resistance to pneumonia even among vaccinated men. Of the 155 cases of pneumonia (all types) developing 1 week or more after vaccination, 133 were secondary to influenza.

9. The death rate for 155 cases of pneumonia (all types) that developed among vaccinated men 1 week or more after vaccination was only 12.2 per cent, whereas the death rate for 327 cases of all types that occurred among unvaccinated troops was 22.3 per cent. The death rate for primary pneumonia among vaccinated troops was 11.9 per cent. Among unvaccinated, it was 31.8 per cent, almost three times as great. On the other hand, the mortality rate in pneumonia secondary to influenza is about the same for the vaccinated and unvaccinated groups.

10. In conclusion, it must be admitted that the results of pneumococcus vaccination at Camp Wheeler have not been so striking as those obtained at Camp Upton in 1918, largely on account of the influenza epidemic; but, although influenza obscured to some extent the effect of pneumococcus vaccination at Camp Wheeler, the results are sufficiently encouraging to justify its further application in civil as well as in military life.

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THE ACTION OF STROPHANTHIN ON THE LIVING CAT'S HEART.*

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PLATES 23 TO 25.

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INTRODUCTION AND HISTORICAL REVIEW.

A review of the pharmacological literature concerning the action of the digitalis bodies reveals a complicated problem and conflicting views. Many difficulties in the way of clear understanding have resulted from the different conditions under which similar phases of this question were investigated, from the impossibility of translating the results of a study of one species to another, and from attempting to transfer the results of the study of one of the digitalis glucosides into terms of another. A prime difficulty consists in the fact that the fate of these drugs within the body is not understood, in respect either to their internal catabolism or to their excretion. This is true partly because much of the experimental work has been done on isolated frogs' hearts perfused in various ways. The interpretation of results from such experiments must be made carefully because they provide no opportunity for essential studies, such as excretion by the kidneys, combination or destruction of the drugs by other tissues, or for observing the influence of the extracardiac nervous control. For these reasons additional knowledge that will help to clarify any part of this problem is of importance, especially if it improves the technique of the clinical administration of digitalis.

Importance of the Concentration of Digitalis and of the Total Amount.—A review of the literature of this subject shows that one of the important problems relating to the digitalis glucosides concerns their mode of action. Does this depend on a physical or a chemical process? Many experiments on isolated frogs' hearts have given evidence that standstill of the ventricle is obtained in the same length of time even when the amount of the perfusion fluid is altered if the concentration of the active principle is kept constant. If the total amount of perfusion fluid is constant, however, the time for standstill will be inversely proportional to the concentration. Straub (1) and later Holste (2), using pure

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strophanthin, came to the conclusion that the action depended on the concentration to which the heart was exposed and not on the total amount of the drug. Grünwald (3), using digitalin and isolated frogs' hearts, found that although the concentration of the drug was the most important factor, the total amount of the solution and therefore of the drug, was of considerable influence in experiments where low dilutions were used. Straub (4) ascribed the discrepancy in Grünwald's results to the fact that while the latter used digitalin, he used pure strophanthin. Weizsäcker (5) agreed with Straub that although the amount of fluid was quite important in low dilutions of digitalin, the amount had no effect on the action of strophanthin. The lowest dilution of strophanthin he used, however, was 0.00025 per cent. Clark (6) and Issekutz (7) found marked differences with digitoxin and only slight, but definite ones with strophanthin when equal concentrations but different amounts were compared. The importance of the problem as to whether digitalis acts because of a certain concentration or because of a certain total amount is appreciated when one realizes that clinically the drug is given, neither to produce standstill of the heart, nor even to produce toxic effects, but rather in amounts so small as to be comparable with those low dilutions in which Grünwald, Clark, and Issekutz found that the total amount of the drug played some part. It is reasonable to suppose that when the drugs are used clinically in such small amounts and in such low dilutions the small amount taken up by the heart and perhaps by other tissues of the body would be sufficient definitely to alter the remaining concentration in the blood stream.

Influence of Temperature and Heart Rate.—There are many factors that influence the effects of the digitalis bodies on the heart. The effect of temperature on the rapidity of digitalis action has been thoroughly studied. Higher temperatures uniformly increase this. Trendelenburg (8) showed that lowering the temperature decreased the effect of strophanthin on perfused frogs' hearts. The difference between given changes in temperature he found to be greater with more concentrated solutions and at the lower temperatures. Gunn (9) found that the effect of strophanthin on perfused rabbits' hearts was more prompt at higher temperatures and thought that this was due to the increased coronary circulation and therefore greater exposure of the heart to the drug. Sollmann, Mendenhall, and Stingel (10) found that the toxicity of ouabain for intact frogs or their isolated hearts increased with the temperature, that this increase was greater per degree at the lower than at the higher temperatures, and that the increase was greater than could be accounted for by the increase in heart rate, which in itself enhances the action of the drug. Weizsäcker (11), Clark (12), and Issekutz (7) noted a more rapid action of the digitalis bodies on frogs' hearts when the rate of contraction as controlled by single induction shocks was increased. Weizsäcker (5) distinguished between the time it took for digitalin to show its first effect on the heart by an increase in the strength of the contractions (*Giftbindung*) and the time to produce standstill (*Giftwirkung*), and observed that the ventricular rate influenced the latter but not the former. Pittenger and Vanderkleed (13),

working with the effects of tincture and fluid extract of digitalis on gold fish, found that although the weight of the fish was not important in determining the time to produce death by a certain concentration of digitalis, changes in temperature made striking differences.

Influence of the General Condition of the Heart and of the Pressure of the Perfusion Fluid.—The general condition of the heart greatly influences the effect produced on it by a given amount of digitalis. Clark (12) showed that strophanthin had a more marked and more rapid effect on perfused hearts if the frog was previously in poor health, or if the heart had been long perfused. Williams (14) and later Krailsheimer (15) found that the degree of pressure to which the frog's heart is subjected in perfusion experiments affects the action of digitalin and helleborein. Clinically it is known that although the mechanism of action of the drug may be the same, the influence of digitalis on diseased hearts may result in a different effect than on normal hearts.

Influence of the Character of the Perfusion Fluid on the Type of Standstill.—There has been some difference of opinion as to the factors that determine the position (systole or diastole) in which a heart will stop under the intoxication of digitalis. The location where the solution is applied is important in this connection. Jacobj (16) and Wybauw (17) found that if the digitalis substance is applied to the outside diastolic standstill results, while if it is applied to the inside systolic arrest occurs. The use of blood serum in the perfusion fluid also alters the subsequent effects of digitalis. Cushny and Gunn (18), for instance, observed that the addition either of homologous or heterologous serum to Ringer solution in perfusing rabbits' hearts caused first a stage of stimulation and later a period of depression in the strength of contraction. The hearts could be restored to a normal condition by returning to the use of Ringer fluid. Gröber (19), experimenting with strophanthidin obtained by Fraser (20) from *strophanthus hispidus*, noticed that the amount of the drug used influenced the result, for whereas 0.8 mg. in 1,000 cc. of perfusion fluid arrested a cat's heart in systole, 0.5 mg. stopped it in diastole. The perfusion fluid consisted of one part of defibrinated calf blood and two parts of Ringer solution. Clark (12) found that dilute concentrations are apt to produce diastolic arrest, and more concentrated ones, systolic arrest. The effect is more marked if the heart is already in a hypodynamic condition, due to prolonged perfusion. Werschinin (21), using Williams' (14) apparatus, concluded that 0.05 mg. of strophanthin (Boehringer) in 50 cc. of Ringer solution or normal saline, arrested frogs' hearts in systole, but lower dilutions in diastole. He added that the presence of one-third the volume of rabbit blood cells or serum would enable the lower dilutions to produce systolic arrest. In this work doubt remains as to whether the pressure in the perfusion system might not have played a part in producing diastolic standstill. Clark (22), on the contrary, found that defibrinated frog blood distinctly diminished the activity of strophanthin on perfused hearts, while the serum alone had no such effect. He thought that the red blood cells absorbed small amounts of the drug.

Influence of Inorganic Salts.—The inorganic salts influence the effects produced by digitalis. A perfusion fluid not containing calcium requires twice the amount of digitoxin to produce standstill that is necessary with the ordinary amount of calcium, according to Clark (6). He also found that the digitalis effect is slightly increased by the absence of potassium salts. Conversely, Burridge (23) found that the presence of digitoxin increased the sensitiveness of the heart to calcium.

Fate of Digitalis Bodies in the Organism.—The study of the fate of digitalis glucosides within the body, *i.e.* their combination, destruction, and the method of their excretion, is still incomplete. Cloetta and Fischer (24) gave 1 mg. of digitoxin to each of ten frogs; at the end of 1 hour all the hearts were arrested. In extracting the ten hearts no digitoxin was found by a method that could detect 0.04 mg. None was found in the hearts of twenty additional frogs although an emulsion of the rest of the bodies, excluding the skins, yielded one-third of the digitoxin injected. Nor was any found in the hearts of rabbits and dogs killed with the drug. But when emulsions of heart muscle were incubated with digitoxin at 37°C. for 4 hours, twice as much digitoxin could be recovered from the sediment, that is from the heart tissue, as from the supernatant fluid. On incubating liver a similar result was noticed. Incubations of this nature therefore did not destroy the drug, for practically the entire amount of digitoxin could be recovered from dog's heart muscle, liver, or brain. In similar incubation experiments of frog's heart, muscle, and liver, Clark (22) likewise found that strophanthin was neither destroyed nor combined with unincubated tissue. The behavior of digitalis bodies under the influence of heart muscle in the living animal and of incubated heart muscle accordingly differs. Cloetta and Fischer also examined the urine and noticed that varying amounts of the drug could be detected; in one experiment, 0.3 mg. of digitoxin, for instance, was recovered 4 hours after 3 mg. had been injected into the vein of a rabbit; in another experiment, 0.8 mg. was found in the urine during 4 days on which 4.9 mg. were injected subcutaneously. In still another experiment, 1 hour and 20 minutes following a large intravenous dose of 5 mg. to a dog, they found none in the heart, 0.8 mg. in the liver, and 3 mg. in the entire blood. Clark (22) in his experiments found part of the injected drug in the blood. He gave 0.02 mg. of strophanthin to each of six frogs subcutaneously; all died in ½ hour, and he found about 0.01 mg., or 8 per cent, in the entire blood. In what physical or chemical state it circulates in the blood is not known, but that it is active has been proved by experiment. Clark (22) has shown that small amounts of strophanthin could be found in the blood of grass snakes 16 hours after subcutaneous injection. The whole blood of frogs, he found, diminished the action of strophanthin. Serum alone or washed corpuscles alone had no such effect. He thought that the red blood cells absorb strophanthin in small amounts but only in the presence of serum. Rabbit red cells, on the other hand, do not absorb strophanthin. But in the absence of satisfactory methods for detecting the digitalis bodies, their

presence and the duration of their action cannot be accurately known. Hatcher (25) tested out nine cats and found that after intravenous injection of 0.3 mg. of ouabain, one showed 32 per cent in the blood stream, one 45 per cent, and seven none at all. Similarly after the injection of 5 mg. of digitoxin in two different cats, none was found in the blood in 4 and 5 minutes.

These experiments show that part of the drug remains in the blood stream for a while at least, that a portion is excreted unchanged by the kidney, and that the fate of the rest is uncertain. The heart, therefore, appears either to utilize very small amounts, or to render the drug unrecognizable after it has been acted upon. Hatcher (26) made similar studies and found that in alcoholic extracts of all the tissue of the rat made 5 minutes after subcutaneous injection, strophanthin could be recovered almost entirely. But if the rat was allowed to live a day, only a portion could be found in the gastrointestinal tract, and none in the other tissues. The tissues of the rat, which are highly tolerant to digitalis, destroy the drug, and part of the drug is reexcreted into the gastrointestinal tract. Hatcher and Bailey (27) also showed that in the duodenal loop of a dog, without producing toxic effects, an amount of strophanthin could be destroyed equal to 10 times the minimum lethal dose administered intravenously. This then is the evidence in favor of the view that animal tissue destroys digitalis bodies.

Perfused Hearts.—The experiments of Cloetta and Fischer, Clark, and Hatcher accordingly show that digitalis substances cannot be recovered from living heart muscle, but can be found if the muscle is incubated. Some indication of the amount of digitalis that is taken up and destroyed by the heart in producing standstill may be obtained from perfusion experiments. Straub (1) perfused the heart of a number of frogs (average weight 68 gm.) with the same solution until it was arrested. Although unable to extract any digitalis from the poisoned hearts, he concluded that a certain amount, probably less than 0.0002 mg., was used up. Clark (6) by a similar technique determined that each frog's heart used up about 0.0013 mg. of digitoxin and 0.00008 mg. of strophanthin. Grünwald (3) and Weizsäcker (5) found that to accomplish this result the equivalent amounts of digitalin were 0.05 to 0.1 mg. Weizsäcker (5) made the important observation that the amount of digitalin that combines with the heart is the same, whether the heart is arrested rapidly by a concentrated solution, or more slowly by a weaker solution. If amounts no larger than these suffice to arrest the heart, the amount of the poison in the perfusion fluid must be several times greater than the amount that is taken up by the heart; *i.e.*; in order to be lethal the minimum amount of strophanthin contained in 2 cc. of fluid must be 0.001 mg. But of this amount of strophanthin, the heart, as far as the experiments show anything, takes up somewhere between 0.0002 mg. and 0.00008 mg. (average 0.00014 mg.). In other words, a much greater amount is necessary than is actually made use of.

Issekutz (7) calculated the concentration of strophanthin within the heart by weighing the heart and determining how much strophanthin was lost from the

perfusion fluid. He found in this way that the concentration was 40 times as great inside the heart as in the perfusion fluid and 15 to 20 times as great for digitoxin. Weizsäcker (5) had previously calculated in a similar way that the concentration of strophanthin within the heart muscle was just equal to that in the fluid, while that of digitalin was 25 times as great within the heart muscle as in the perfusion fluid. But careful analysis of his figures shows an error in his calculation for he did not compare the lowest dilution of strophanthin in the perfusion fluid that can cause standstill with the concentration in the heart. This would bring the figure to about 20 and would then agree with the findings of Issekutz. It must be recognized in this connection that when a diminution of the drug is found in the perfusion fluid, it need not be in the heart, as has been mentioned above. It is more probable that it is destroyed by the heart and is no longer recognizable, and that the concentration of the active principle in the perfusion fluid is slightly diminished but always greater than in the heart.

There are facts that point to a selective action of the digitalis bodies on the heart, although other body tissues seem to be affected by the drug. Clark (22) found, for instance, that 0.0005 mg. per cc. of strophanthin was required to produce standstill of the heart, 0.1 mg. per cc. to produce vasoconstriction of the leg or contraction of an isolated stomach ring, and 1 mg. to kill voluntary muscle. And Schliomensun (28) found that alcoholic phosphatids, which possess an especial capacity for combining chemically with the digitalins, could be isolated from the hearts of the human cadaver and of the dog while the corresponding fractions obtained from the skeletal muscles had no such affinity.

Action by Physical or Chemical Processes.—The apparent dependence of these drugs on their concentration has led to the view that their mode of action is a physical one at the surface of the muscle. There are facts, however, that point rather to a chemical change in the heart. Straub (1) showed in perfusion experiments that, if strophanthin solutions were removed and the heart was washed, it revived; recovery did not take place if washing was delayed too long. In the latter event the action was not reversible; *i.e.*, the action of the drug is physical to a certain point only. Issekutz (7) similarly found that the heart frequently could not be revived, even if washed before showing a toxic effect. Clark (6) perfused frogs' hearts with 3 cc. of a solution containing 0.01 mg. of digitoxin. He found, like Straub, that if the heart was washed in 8 minutes, it died subsequently in systole, but if washed in 5 minutes, standstill was prevented.

The analogies which have been made use of in this discussion are numerous. It is impossible, however, to interchange apparent equivalents in a problem as complex as that of the action of the digitalis glucosides. The following facts illustrate this point. The subcutaneous minimum lethal dose of strophanthin is 30 times greater for a rat than for a rabbit and 20 to 40 times the concentration is needed in the perfusion fluid to arrest the isolated heart. On the other hand, while the intact grass snake is 30 times more tolerant to a subcutaneous injection of strophanthin than is the frog, the isolated heart is 1,000 times more

tolerant. Another instance in which simple substitution would lead to error occurs in determining the relative toxicity of the substances for a certain concentration and inferring that the same relation holds true for other concentrations. Issekutz (7), for instance, shows that 5 cc. of 0.1 per cent digipuratum is equivalent to 0.001 per cent strophanthin, *i.e.* a ratio of 1: 100, but 5 cc. of 0.03 per cent digipuratum is equivalent to 0.0000625 per cent strophanthin, a ratio of 1: 500.

The foregoing review indicates how difficult it is to resolve some of these factors. The more important ones are temperature, rate, the general condition of the heart, the pressure of the perfusion system, and the organic and inorganic constituents in perfusion fluids. Animals differ widely; and their reactions are not constant when the mode of administration is changed or when different glucosides are compared. The experiments often yield contradictory results. Careful analyses sometimes uncover the cause. Occasionally, however, the lack of harmony cannot be explained. The absence of a chemical test for digitalis renders it impossible to make any but general statements about excretion, destruction, and combination of the drug by the living organism. But this review indicates in part wherein the difficulties of the digitalis problem lie. They are comprised principally in the diversity of the methods employed, and in the failure to appreciate that results obtained so variously can scarcely serve as the basis of satisfactory generalization. There is no intention to obscure the value in themselves of the important contributions which have so far been made. But with the means now at our disposal and as the result of the experience now accumulated it becomes important that certain requirements be observed in the plan of the experiments in order that the results may be useful in laying the foundations for clinical practice. In the experiments now reported cats have accordingly been employed because of their relatively uniform reaction to the digitalis bodies and because the same animal can be used for a number of separate experiments. Repeated use in this way made it possible to avoid the danger of lack of uniform results due to varying susceptibility even in animals of the same species. The technique of electrocardiographic registration permitted the performance of the experiment so that operative procedures on the heart itself were not undertaken and disturbances from this source were avoided. This technique in addition afforded the opportunity to study in detail the influence of time on the results of administration.

Method.

Cats were selected in preference to other laboratory animals because their reaction to digitalis is more like that of the human subject. Crystalline g-strophanthin was used because of its chemical purity and to avoid the confusion from interaction of the principal glucosides if the whole digitalis leaf is used. A 1 per cent alcoholic solution was prepared and kept, and from this a 0.001 per

cent solution was made up every few days. This was used in the intravenous injections. There was no indication that the stock solution deteriorated during the period of the experiments. The solutions were kept sterile. The cats were etherized during the operative procedure. Afterward as little ether was used as was necessary to keep the animal sufficiently quiet for taking satisfactory electrocardiograms. But in consequence cats were occasionally lost by an overdose of ether, although it was always possible to distinguish by the electrocardiograms an ether death from one produced by strophanthin. During some experiments no additional ether was given after the injections were started. The strophanthin solution flowed from a graduated burette into the right saphenous vein.

Use of the Electrocardiograph to Detect the Toxic Effect.—The string of the galvanometer (Edelmann model) was connected with the right fore and left hind leg (Lead II). Electrocardiograms were taken before the injection of the drug and frequently during the course of the experiment. It was observed by Halsey (29) that if 40 to 60 per cent of the minimum lethal dose[1] of strophanthin is given intravenously extrasystoles appear. He fixed no time interval during which he gave strophanthin, although he states that 30 per cent was given in about 15 minutes. He gave the rest at irregular intervals. It is, in fact, impossible to find in any previous work determinations of what portion of the minimum lethal dose, given in a definite interval of time, is required to produce extrasystoles. The smallest amount required to cause ectopic premature beats we propose to call the "minimum toxic dose." The only reliable method of determining the minimum toxic dose is electrocardiographic. This method excludes the possibility of misinterpreting the nature of the irregularity. It also dispenses with the necessity of exposing the heart or disturbing the animal. It is

[1] The minimum lethal dose of crystalline strophanthin for cats as determined by Hatcher and Brody (Hatcher, R. A., and Brody, J. G., *Am. J. Pharm.*, 1910, lxxxii, 360) is 0.1 mg. per kilo of body weight if given intravenously and almost continuously over a period of 1½ hours. This was confirmed by Jamieson (Jamieson, R. A., *J. Exp. Med.*, 1915, xxii, 629), who used the same active principle as that in the experiments reported here.

more reliable to use the occurrence of ventricular ectopic beats as the index of toxic action than the vagal effects, such as slowing of the heart rate, lengthening of the auriculoventricular (P–R) interval, or the development of exaggerated sinus arrhythmia.[2] Electrocardiograms of typical toxic reaction with subsequent death in ventricular fibrillation are shown in Fig. 1. In a very few instances the toxic effect was similar to those described by Cohn (30) and Christian (31) as resulting from digitalis intoxication in man. The form of the ventricular complex continually changes; an example of this reaction is shown in Fig. 2. Injections were made every 6 minutes and frequently, on approaching the toxic dose, the interval was diminished to 3 minutes. The method of interrupted administration introduces an error of from 3 to 6 per cent in determining the minimum toxic dose, for by giving injections every 6 minutes at the rate of 60 per cent of the minimum lethal dose in an hour, 6 per cent of the minimum lethal dose is given with each injection. The final 6 per cent that is administered, which produces the toxic effect, might have been excessive by 5 per cent, for an additional 1 per cent might have produced the same toxic effect. This error cannot be avoided by giving an absolutely continuous injection, for in that case, the amount of drug given during the last few minutes before the toxic effect appears might have been unnecessary.

Frequency of Spontaneous Nodal Rhythm in Cats.—In the interpretation of cardiac irregularities in cats, attention is called to the great frequency of spontaneous nodal rhythm (Fig. 3). It would be almost impossible to recognize this alteration in the heart's mechanism by any means other than electrocardiographic. Of seventeen animals (Table I), nine showed nodal rhythm one or more times during the various experiments. Some cats showed it several times, others only once or twice. Eight showed it before strophanthin was given in that particular experiment. Some of them also showed it during the early part of the administration, when very small amounts had been given, one after 8 per cent of

[2] Although more reliable, we do not recommend the occurrence of ventricular ectopic beats as the sign of toxic action in clinical medicine, as earlier signs are available.

TABLE I.

Experiment No.	Date.	Weight.	Rate of injection.		Minimum toxic dose (amount to produce toxic effect).	Per cent of minimum lethal dose required to produce effect calculated from.		Time for effect.	Type of effect.*	Spontaneous irregularity.
			Per cent of minimum lethal dose.	Time.		Original weight.	Actual weight.			
	1917	*kg.*			*cc.*					
1 a	Jan. 8	3.00	40	1 hr.	Leak in cannula.	?	?	104 min.	V. E. C.	
b	" 10	2.65	40	2 hrs.	13.8	46	52	133 "	"	N. R.
c	" 12	2.40	40	½ hr.	8.4	28	35	23 "	"	
d	" 16	2.05	40	¼ "	15.2	50	74	20 "	"	
2 a	Jan. 8	4.05	40	1 hr.	Leak in cannula.	?	?	134 min.	V. E. C.	
b	" 11	3.65	40	2 hrs.	16.0	40	43	117 "	F. P. V. C.	
c	" 12	3.50	40	½ hr.	16.0	40	46	28 "	"	
d	" 16	3.45	40	¼ "	25.5	62	70	24 "	V. E. C.	N. R.
3 a	Jan. 9	3.35	40	1 hr.	13.4	40	40	56 min.	V. E. C.	N. R.
b	" 11	3.35	40	2 hrs.	15.6	47	47	141 "	"	"
4 a	Jan. 16	3.65	40	1 hr.	22.0	60	60	95 min.	F. P. V. C.	N. R.
b	" 20	3.40	40	¼ "	16.8	46	49	20 "	C. V. C.	"
5 a	Jan. 18	3.00	40	1 hr.	18.0	60	60	88 min.	V. E. C.	
b	" 22	2.70	40	¼ "	19.2	64	71	23 "	"	
c	" 26	2.65	40	2 hrs.	12.0	40	45	117 "	F. P. V. C.	N. R.
6 a	Jan. 23	3.00	50	1 min.	15.0	50	50	13 min.	Tracings lost.	
b	" 29	2.80	50	1 hr.	10.5	35	38	38 "	C. V. C.	
c	Feb. 2	2.35	50	2 hrs.	9.0	30	38	70 "	F. P. V. C.	
7 a	Jan. 24	2.25	60	1 min.	13.5	60	60	∞	0	
b	" 30	2.00	60	1 hr.	11.7	52	59	49 min.	F. P. V. C.	
c	Feb. 5	1.65	52	1 min.	11.7	52	71	12 "	C. V. C.	

8 a	Jan. 25	2.95	60	1 hr.	18.5	63	63	76 min.	F. P. V. C.	
b	" 30	2.50	60	¼ "	15.2	52	61	13 "	V. E. C.	
c	Feb. 6	2.00	60	2 hrs.	11.7	40	59	96 "	F. P. V. C.	N. R.
9 a	Feb. 7	4.40	60	¾ hr.	19.3	44	44	11 min.	C. V. C.	
b	" 13	3.45	60	2 hrs.	20.8	47	60	91 "	O. P. V. C.	Bigeminy.
c	" 19	3.15	60	1 hr.	15.6	35	49	35 "	V. E. C.	
d	" 23	2.90	60	4 hrs.	18.9	43	65	173 "	F. P. V. C.	
10 a	Feb. 8	3.20	60	2 hrs.	23.0	72	72	143 min.	F. P. V. C.	
b	" 13	2.90	60	½ hr.	22.8	71	78	36 "	?	
11 a	Feb. 9	3.25	60	2 hrs.	23.3	72	72	143 min.	O. P. V. C.	
b	" 14	2.90	60	½ hr.	20.6	64	71	35 "	V. E. C.	
12 a	Feb. 14	3.00	60	¼ hr.	14.4	48	48	9 min.	V. E. C.	N. R.
b	" 20	2.45	60	½ "	10.8	36	45	17 "	"	"
c	" 24	2.35	60	1 "	12.6	42	54	41 "	"	"
d	" 28	2.15	60	4 hrs.	10.8	36	50	122 "	"	"
13 a	Feb. 15	2.65	60	1 hr.	6.4	24	24	23 min.	O. P. V. C.	
b	" 20	2.25	60	¼ "	6.4	24	28	5 "	"	N. R.
14 a	Feb. 16	3.40	60	4 hrs.	18.0	53	53	214 min.	F. P. V. C.	
b	" 21	2.95	60	1 hr.	18.0	53	61	48 "	V. E. C.	N. R.
c	" 26	2.50	60	¼ "	21.3	63	85	17 "	F. P. V. C.	
15 a	Mar. 2	4.70	35	2 min.	16.4	35	35	16 min.	V. E. C.	
b	" 7	4.55	60	1 hr.	16.8	36	37	38 "	F. P. V. C.	
16 a	Mar. 5	3.15	30	1 min.	13.1	42	42	42 min.	C. V. C.	
			60	2 hrs.						
b	" 9	2.75	30	1 hr.	15.2	48	55	45 "	F. P. V. C.	
17 a	Mar. 8	2.85	30	1 min.	14.4	51	51	83 min.	V. E. C.	
			60	2 hrs.						
b	" 19	2.45	39	1 min.	11.0	39	45	9 "	F. P. V. C.	

* V. E. C. indicates ventricular ectopic tachycardia; F. P. V. C., frequent premature ventricular contractions; O. P. V. C., occasional premature ventricular contractions; C. V. C., changing ventricular complexes; N. R., nodal rhythm.

the minimum lethal dose had been injected. Two others, No. 9 b and one not included in Table I, showed spontaneous bigeminy, every other beat being a premature ventricular systole, before any strophanthin was given. Three cats had nodal rhythm in the first experiments (marked "a" in Table I) on the respective animals. There is no reason to believe that the cats had taken any drug that might have caused this rhythm. None of them had previously been experimented upon. The high incidence of spontaneous nodal rhythm is pointed out because it may have a bearing on experiments concerned with this arrhythmia as in the work of Lewis, White, and Meakins (32).

Independence of the Minimum Toxic Dose of the Speed of Administration of the Drug.—In the experiments to be described we compare the effects of rapid with slow administration in the same animal. The method is designed to yield information on the problem of whether strophanthin acts by means of its concentration or by virtue of the total amount present. The volume of the blood remains constant. By injecting it rapidly strophanthin is distributed in the circulation quickly, and the heart, therefore, is immediately exposed to a high concentration; when the injections are slow, the heart is exposed to a lower concentration for a given length of time, for a smaller amount of the active principle is contained in the same volume of blood. Thus the rate of injection determines the concentration in the blood stream. Some cats served for four separate experiments, others for three, and some for only two. The method of administration was changed from cat to cat; some received the rapid injection first and later the slow one. The reverse method was employed in others. The original minimum lethal dose was calculated on the original weight, and in later experiments the same dose was used, although the weight had diminished. This will be discussed below. In this way one could determine whether the animal's later condition as a result of the action of the drug, or the long experimentation, or the administration of ether, altered the amount of the drug required to produce the same toxic effect. There was no indication that any striking change in the animal's susceptibility to the drug took place. In the first experiments the solution (0.001 per cent)

was given at such a rate that 40 per cent of the minimum lethal dose was injected in an hour. The same cat, usually 4, though sometimes more and occasionally fewer days later, when free of the drug was subjected to the same procedure but the rate of injection was 40 per cent in 2 hours, or 40 per cent in $\frac{1}{2}$ hour, etc. The cat was considered free from the effects of the drug because Hatcher (33) showed that 24 to 48 hours following a toxic but nonlethal intravenous injection of strophanthin, a similar dose is required to produce a similar result. In the later experiments 60 per cent of the minimum lethal dose was given, because it caused toxic effects more regularly. The injections were stopped when the toxic effect occurred. This could usually be detected by watching the shadow of the galvanometer string, because of the abnormal form of the ectopic beat, and the occurrence of an irregularity. Occasionally it was difficult to be certain whether a toxic effect was produced; then electrocardiograms were taken frequently. When the films were developed, the intoxicating dose was estimated.

EXPERIMENTAL.

The experiments are divided into three groups: those in which was studied the influence of the speed of injection on the amount of strophanthin required to produce a toxic effect; those in which single injections were made to obtain the minimum toxic and lethal dose and to compare this with the amount required when the rate of injection is slow; and transfusion experiments made to obtain additional information on the concentration of the drug in the blood.

Group 1.—Group 1 (Table I) contains seventeen experiments. In these was studied the influence of the speed of injection on the amount of strophanthin required to produce a toxic effect. The animals frequently lost weight. Whether this resulted from loss of appetite, or from prolonged etherization and the operative procedure, or from strophanthin intoxication is not known. The animal usually ate nothing after the operation and very little the next day. On the 4th day it was usually operated upon again. The loss in weight was disturbing in calculating the minimum lethal

dose. It seemed correct, however, to base the minimum lethal dose on the original, rather than on the actual weight (both of which were ascertained), for it is not likely that the heart lost weight during these experiments as rapidly as the whole body.[3]

There are (Table I) distinct individual variations among cats in susceptibility to toxic doses of strophanthin. It required, for instance, 72 per cent of the minimum lethal dose, given at the rate of 60 per cent in 2 hours, to produce toxic effects in Cats 10 a and 11 a, while only 24 per cent, at the rate of 60 per cent in 1 hour, was required in Cat 13 a. The difference in rate of administration will be shown not to influence the minimum toxic dose. Other animals required amounts between these limits. The significant point is that in the second, third, and fourth injections in the same animal, although slight variations in the minimum toxic dose occur, they do not depend on the speed of administration. This conclusion is warranted from the average of the experiments (Table II) and from an analysis of the individual ones.

TABLE II.

Average.	Per cent of minimum lethal dose required to produce toxic effect, calculated from.	
	Original weight.	Actual weight.
Of all in Table I	47.4	53.7
" first injections ("a" in Table I)	51.6	51.6
" all subsequent injections	48.7	54.8
" last injections	46.5	56.9
" 1 hr. first injections	49.4	49.4
" ¼ " injections	50.3	58.9
" ½ " "	47.8	55.0
" 1 " "	45.5	49.5
" 2 hrs. "	45.3	52.0
" 4 " "	44.0	56.0

The average minimum toxic dose of strophanthin in all the experiments (Table II) was 47.4 per cent of the minimum lethal dose,

[3] In a fasting cat the heart lost only 2.6 per cent of its weight, while the liver lost 53.7 per cent, and the whole body over 1,000 gm. (Howell, W. H., A text book of physiology for medical students and physicians, Philadelphia and London, 5th edition, 1913, 914).

if the original weight, and 53.7 per cent if the actual weight before each experiment is used as the basis of calculation. The second figure is higher than the first because of the animal's loss in weight. The average of all first experiments before loss of weight has occurred is 51.6 per cent. The average in the first experiments in which injections were given in an hour is 49.4 per cent. In these the influence of the loss of weight due to experimentation is entirely avoided. On the whole, there are no serious discrepancies in the various averages calculated in Table II. They show that the speed of injection does not influence the minimum toxic dose. The average minimum lethal dose in the short 15 minute experiments was 50.3 per cent; in the longer 4 hour ones 44.0 per cent. In those of intermediate length the percentages increased as one might expect. One might infer from the figures based on the original weights that the slower method requires slightly less strophanthin than the rapid, but the difference is probably not significant. An explanation for it may exist in the fact that in the slower method the dose given just before the toxic effect is produced, has acted a longer time on the heart; while in the rapid experiments an excessive amount was probably injected. The injections, for instance, were made at the rate of 4 cc. every 3 minutes. When the end-point was approached 1 cc. might have been sufficient to cause a toxic effect. Errors of this kind are smaller in the slower experiments because a smaller amount is given every 6 minutes.

Two experiments, Nos. 9 and 12 (Table I), illustrate these points. Four injections at different rates were given in each case. In No. 9 the first injection was given at the rate of 60 per cent in 15 minutes, and the toxic effect (minimum toxic dose) was produced with 44 per cent of the minimum lethal dose. 6 days later the same cat received an injection at the rate of 60 per cent in 2 hours and showed toxic manifestations with 47 per cent; the third injection, 6 days later, was given at the rate of 60 per cent in 1 hour and the minimum toxic dose was 35 per cent; the final one was given 4 days later at the rate of 60 per cent in 4 hours, and the minimum toxic dose was 43 per cent. Similar results were obtained in the four injections in No. 12. The rate at which the

injections were given was also 60 per cent of the minimum lethal dose in varying intervals of time. The variations were 48 per cent for 15 minutes, 36 per cent for 30 minutes, 42 per cent for 1 hour, and 36 per cent for 4 hours. In these instances, then, the minimum toxic dose is independent of the rate of injection. To test the point further, the rate of injection was changed in several instances during the same experiment. If a cat at first was given 30 per cent in 1 minute, injection was continued after waiting 15 minutes at the rate of 60 per cent in 2 hours. The toxic effect again was produced after a certain total amount had been given.

Group 2.—Further evidence on the relation of rate of injection to amount injected was obtained from a series in which large single injections were made in 1 or 2 minutes. It is, of course, impossible in a given animal to forecast the minimum toxic dose.

TABLE III.

Per cent of minimum lethal dose given in 1–2 min.	25	30	35	40	50	55	60	70	75	80	90	100
Result.	4 N.*	4 N.		1 N.	2 N.		1 N.					
	1 T.	1 T.	1 T.	1 T.	1 T.	1 T.	1 T.	2 T.		1 T.	1 T.	1 T.
									1 F.	1 F.	1 F.	2 F.

* N. indicates negative result; T., toxic effect; F., fatal.

It was therefore necessary in a series of animals to obtain it by trial. The averages then served for comparison. There were twenty-nine cats. They received single injections of amounts varying from 25 to 100 per cent of the minimum lethal dose. There was, as was expected, great variation in the minimum toxic dose, for some cats showed effects from 25 per cent, while others required 60 per cent of the minimum lethal dose (Table III). Of five experiments in which 25 per cent was given only one was toxic. One cat in five was found to be intoxicated by injections of 30 per cent; one in two experiments in which 40 per cent was injected; one in three 50 per cent injections, and one in two 60 per cent injections. All injections over 60 per cent of the minimum lethal dose caused toxic effects and many were also fatal. There was also some variation in the minimum lethal dose, for 75 per cent

was fatal to one cat while 100 per cent was not to another. It is difficult to calculate an average toxic dose from such experiments, but in general these figures agree with those of Table II. If flooding the circulation suddenly with a large dose of strophanthin were more toxic than giving the same dose continuously over an hour or two, one would expect a greater percentage of toxic and lethal effects than were obtained. When 30 per cent, for instance, was given, only one of five showed an effect.

These results throw doubt on the conclusion that strophanthin produces its effects in accordance with the concentration of the drug to which the heart is exposed. For the differences in the speed of administration must alter its concentration in the blood stream, the blood volume probably remaining constant. So far, then, the toxic effect appears to be produced when a given total amount has been injected into the blood. An objection to this conclusion may, however, be offered; strophanthin, no matter at what speed administered, may be supposed to remain inert in the blood stream until a certain amount has been given, to provide a concentration sufficient for activity. When the required concentration is reached the heart is intoxicated. The objection is hardly valid, for there is evidence that even before the toxic effects appear strophanthin affects the heart. The therapeutic effects, for instance, of the drug on the heart are obtained by subtoxic doses.

Cohn, Fraser, and Jamieson (34) have shown that the heart muscle may be affected by digitalis before toxic symptoms are produced. Robinson and Wilson (35) show that to bring about T wave changes in the electrocardiogram of cats only 23.9 per cent of the lethal dose is necessary. Gros (36), using very dilute solutions of strophanthin on the perfused frog's heart, produced intermittent diastolic pauses without any other toxic results. These observations suggest that throughout the experiments the cats' hearts take up strophanthin, the toxic symptoms appearing when a certain total amount has been taken up.

Group 3.—Transfusion experiments were undertaken to obtain information on the amount of the drug actually present in the blood.

Experiment 18.—Cat A, weight 4,150 gm. was given 0.1 mg. of strophanthin at 11.05 and 0.1 mg. at 11.15 a.m. At 11.20 ventricular ectopic tachycardia appeared. At 12.50 p.m., 75 cc. of blood were taken from left carotid artery into

citrate solution. From a second cat, B, weight 4,250 gm., (the minimum toxic dose having previously been found to be 66 per cent of the minimum lethal dose), 70 cc. of blood were withdrawn at 12.50 from the left carotid artery. At the same time 70 cc. of strophanthinized blood from Cat A were transfused into the right saphenous vein of Cat B. At 1.10 the injection of strophanthin intravenously was started at the rate of 0.03 mg. every 6 minutes. After 0.12 mg. had been given, ventricular ectopic tachycardia was noticed. The minimum toxic dose equalled 28 per cent of the minimum lethal dose. Death occurred after 0.225 mg. had been given. This equalled 53 per cent of the minimum lethal dose.

The low minimum toxic dose and the low minimum lethal dose indicate that some strophanthin must have been contained in the blood of Cat A, for it took much less additional strophanthin to produce either a toxic or a fatal result in Cat B than we know would otherwise have been required. It is impossible to calculate how much of the drug the transfused blood of Cat A contained, but the result makes it reasonable to say that 1 hour and 45 minutes after the toxic effect had been produced the blood of Cat A contained at least a moderate amount of strophanthin.

Experiment 19.—Cat A, weight 4,400 gm., was given 0.1 mg. of strophanthin at 10.26 and 0.1 mg. at 10.36 a.m. At 10.43 a toxic effect occurred. At 11.55 an added 0.2 mg. was given intravenously. At 12.05 p.m. 100 cc. of blood were taken from the left carotid artery. Then from a second cat, B, weight 3,300 gm. (the minimum toxic dose for which having previously been found to be 67 per cent of the minimum lethal dose), 70 cc. of blood were removed at 12.40. At 12.42, 85 cc. of blood from Cat A were transfused into the left saphenous vein of Cat B. Then at 1.12, 0.026 mg. of strophanthin was given every 3 minutes. A toxic effect was produced with 0.147 mg., the equivalent of 45 per cent of the minimum lethal dose, and death occurred after 0.303 mg. had been injected. This equalled 92 per cent of the minimum lethal dose.

This experiment also indicates that a certain amount of strophanthin was carried over in the blood from Cat A, for the minimum toxic dose for Cat B when first tested was 67 per cent of the minimum lethal dose and after transfusion was only 45 per cent.

Experiment 20.—Cat A, weight 2,900 gm., was given on Mar. 31 at 10.35 a.m., 0.1 mg. of strophanthin; at 10.53, 0.05 mg.; at 10.57 a toxic effect was observed. At 11.40, 70 cc. of blood were taken from the left femoral artery. At 11.45, 75 cc. of normal saline solution were given. At 12 m. 25 cc. of citrated blood from Cat B were injected; at 12.37 p.m., 0.05 mg. of strophanthin was given; at 12.42, 0.05 mg., and at 12.47, 0.02 mg. The fatal dose was 0.27 mg.; *i.e.*, 93 per cent of the minimum lethal dose.

On Mar. 27, the minimum toxic dose of Cat B, weight 2,600 gm. was found to be 77 per cent of the minimum lethal dose. On Mar. 31, at 11.45, 50 cc. of blood were taken from the right carotid artery. At 11.52, 65 cc. of strophanthinized blood from Cat A were transfused into Cat B. At 1.17, 0.063 mg. and at

1.23 an additional 0.063 mg. of strophanthin were injected. At 1.45 a toxic effect was produced. In this experiment, therefore, a toxic effect was produced with 48 per cent of the minimum lethal dose. At 1.48, 0.031 mg., and at 1.51, 0.031 mg. of strophanthin were added. The cat died at 1.57 after taking 72 per cent of the minimum lethal dose.

Cat A, weight 2,900 gm.		Cat B, weight 2,600 gm. (minimum toxic dose previously found to be 77 per cent of minimum lethal dose).	
a.m.–p.m.		*a.m.–p.m.*	
10.35	0.1 mg. of strophanthin.		
10.53	0.05 " " "		
10.57	Toxic effect.		
11.40	Bled 70 cc.		
11.45	75 cc. of saline solution intravenously.	11.45	Bled 50 cc.
		11.52	65 cc. of strophanthinized blood of Cat A.
12.00	25 cc. of blood from Cat B.		
12.37	0.05 mg. of strophanthin.		
12.42	0.05 " " "		
12.47	0.02 " " "		
12.51	Died.		
		1.17	0.063 mg. of strophanthin.
		1.23	0.063 " " "
		1.45	Toxic effect.
		1.48	0.031 mg. of strophanthin.
		1.51	0.031 " " "
		1.57	Died.

Minimum toxic dose for Cat A = 52 per cent of the minimum lethal dose. Lethal dose for Cat A = 93 per cent of the minimum lethal dose. Minimum toxic dose for Cat B = 65 cc. of strophanthinized blood of Cat A plus 48 per cent of the minimum lethal dose. Minimum lethal dose for Cat B = 65 cc. of strophanthinized blood of Cat A plus 72 per cent of the minimum lethal dose.

From Cat A, then, 43 minutes after the toxic effect was produced about half[4] of the blood was removed (70 cc.). Some strophanthin must have been removed with the blood; however, 93 per cent of the minimum lethal dose was required to cause death. Cat B, which required 77 per cent of the minimum lethal dose to produce toxic effects in the preliminary test, was toxic after the injection of the 65 cc. of blood taken from Cat A, plus 48 per cent of the minimum lethal dose. It died after 72 per cent was injected. This amount was less than the minimum toxic dose on the first occasion.

[4] Calculated on the basis that 5.5 per cent of cat's weight is blood. (Ellenberger, W., and Scheunert, A., Lehrbuch der vergleichenden Physiologie der Haussäugetiere, Berlin, 1910.)

Experiment 21.—The minimum toxic dose of Cat A, weight 3,850 gm., was found to be 72 per cent of the minimum lethal dose. On Apr. 3, at 11 a.m., 0.092 mg. of strophanthin was injected, and at 11.15 an additional 0.08 mg. (47 per cent). No toxic effect was produced. At 12.42 p.m., 90 cc. of blood were withdrawn from the left femoral artery. At 12.46, 45 cc. of blood taken from a normal cat were transfused. At 12.55, 25 cc. of normal saline solution were given. Beginning at 1.06, 0.046 mg. of strophanthin was given every 6 minutes until death. The toxic effect occurred after an additional 34 per cent of the minimum lethal dose (total 81 per cent) and the lethal dose after 116 per cent was injected.

This experiment shows in a somewhat different manner from the previous transfusion experiments that an appreciable amount of strophanthin must have been removed in bleeding, for although 47 per cent of the minimum lethal dose was given without producing a toxic effect, 34 per cent more had to be given after the bleeding to cause the toxic symptoms. If all the strophanthin had been bound either to the heart or to the other tissues so much would hardly have been required. The fatal dose was in excess of the expected amount if all had been retained in the body. Of the four transfusion experiments No. 21 was the only one which showed a larger minimum toxic dose than was previously found in the corresponding cat, and it was also the only one in which the minimum lethal dose was more than 100 per cent.

These results are in accord with the idea that strophanthin remains in the blood stream, for while in Experiments 18 to 20, strophanthinized blood from other cats was added, in Experiment 21 strophanthinized blood was removed. These transfusion experiments are imperfect and do not permit an accurate calculation of the concentration of the drug in the blood stream. They show, however, that an appreciable amount is present in the peripheral circulation an hour or more after intravenous administration, whether a toxic effect is produced or not.

Strophanthin almost invariably caused death by fibrillation of the ventricles; respiration continued longer. There is no way of preventing this or of stopping it after its inception. If a fatal issue depends on the presence of strophanthin in the blood, the withdrawal of blood should prevent or delay death. An attempt was accordingly made after injecting 100 per cent of the minimum lethal dose to prevent ventricular fibrillation by bleeding the animals as soon as the toxic effect was observed. This procedure failed in three experiments. Whether a fatal outcome would

occur if the drug were given slowly was not ascertained. Ether never caused fibrillation of the ventricles; respiration stopped, but the electrocardiogram showed the presence of heart block for a long time. Massaging the heart when the ventricles fibrillate, frequently delayed death for ½ hour. The pupils remained small, and respiration continued. If massage was stopped the pupils dilated and respirations became labored or ceased; on resuming massage the pupils again became small, and the respirations more easy. In the absence of any other treatment massage of the heart offers a possible method of treating fibrillation, although the chances are against a favorable outcome. In all but one instance the cat finally died.

The outcome of this exceptional case, Cat 7 b, is important, for no other case of recovery from digitalis poisoning in an experimental animal has been recorded. This cat was given 0.013 mg. of strophanthin every 6 minutes; the ether cone was kept in place but no ether was added after the injections began. In 49 minutes, when 52 per cent of the minimum lethal dose had been given, extrasystoles were observed (Fig. 4); the injections were stopped, but 10 minutes later the ventricles fibrillated. Electrocardiograms were obtained at frequent intervals during this time. The heart was massaged without opening the chest. For a time the galvanometer string was practically motionless, then unusual deflections were seen, and later a return to typical ventricular fibrillation; finally ventricular tachycardia developed. The breathing improved; the pupils, which were at times markedly dilated, became small, and the cat recovered. The last tracing (Fig. 4), taken 22 minutes after the onset of ventricular fibrillation, shows a normal cardiac mechanism except for delayed conduction (P–R) time, 0.16 second. 6 days later the animal was used again for an experiment.

A number of details deserve notice. (1) In one experiment (No. 20) ventricular ectopic tachycardia did not appear until 90 per cent of the minimum lethal dose had been injected. The warning is therefore expressed that intoxication may be present, without the appearance of extrasystoles.

(2) In fractional administration irregularities usually occurred ½ to 4 minutes after the last injection. Their duration was usually longer than 30 minutes. Durations of 30 and 50 minutes were observed in two cats, and in seven other cats, of ½ to 4 hours. After single large doses, irregularities began in 2 to 15 minutes. Death occurred in 9 to 30 minutes.

(3) Numerous fatalities in patients have resulted from the intravenous administration of strophanthin, but most of them have occurred when this drug was given to patients who recently had taken digitalis, or when large doses were repeated on the same day. It is

TABLE IV.

Experiment No.	Method of administration.	Minimum toxic dose.	Minimum lethal dose.	Margin of safety.	
		per cent	*per cent*	*per cent*	
3 b	40 per cent of minimum lethal dose in 2 hrs.	47	70	23	(23)*
22 c	40 per cent in 1 min. was toxic; 2½ hrs. later irregularity persisted and 20 per cent was fatal.	40	60	20	(25)
5 c	40 per cent of minimum lethal dose in 2 hrs.	40	78	38	(43)
6 c	50 per cent of minimum lethal dose in 2 hrs.	30	45	15	(19)
7 c	52 per cent in 1 min. was toxic; 1 hr., 20 min. later irregularity had disappeared and 20 per cent in 1 min. was fatal.	52	72	20	(27)
8 c	60 per cent in 2 hrs.	40	55	15	(22)
23 a	70 per cent in 2 min. was toxic; 1 hr., 50 min. later heart again regular and 30 per cent in 1 min. was fatal.	70	100	30	(30)
10 b	60 per cent in ½ hr.	71	89	18	(20)
12 d	60 per cent in 4 hrs.; waited 1 hr. after toxic effect; heart irregular then; 6 per cent in 1 min. was fatal.	36	42	6	(8)
20 a	60 per cent in ½ hr.	54	96	42	(42)
21 a	60 " " " ½ "	72	90	18	(18)

*All percentages were calculated on the basis of the original weight of the animal. The figures in parentheses are calculated on the actual weight and are slightly higher.

impossible from these experiments to make recommendations for the administration of strophanthin to man. They show that a margin of safety exists, represented by the difference between the minimum lethal dose and the minimum toxic dose. This difference (Table IV)

varied between 6 and 42 per cent of the minimum lethal dose. To predict the toxic dose is, therefore, impossible. The dose, for example, that was fatal to Cat 12 was not even toxic for Nos. 3, 10, 20, and 21. To foretell the toxic dose in patients is probably equally difficult. But since these experiments show that the same results can be obtained from slow as from rapid injections, a fractional method of administration suggests itself. If, for instance, 0.5 mg. given in a single injection were fatal, 0.2 mg. might be given followed by 0.1 mg. repeated every half hour until 0.5 mg. is given. It may be possible to regulate the procedure by watching for signs of intoxication, either by auscultating for irregularities or by taking graphic records, to notice changes in the T waves or the P–R time. Under these conditions, giving an excess of more than 0.1 mg. is avoided. Treatment should not be repeated within 24 hours. Vaquez and Lutembacher (37) have reported almost 2,000 intravenous injections of ouabain without harm or fatality.

DISCUSSION AND SUMMARY.

The review of the literature relating to experiments on the action of the digitalis glucosides shows that care must be exercised in estimating the significance of the results published. The perfusion of isolated hearts obviously cannot take into account the question of excretion of the drug or its combination with other tissues. Nor can perfusion experiments take into account the influence of the extracardiac nerves. Vagotomy alone, as Macht and Colson (38) have shown, has a marked influence on the result. And Richards and Wood (39) have shown that the effect of the intravenous injection of strophanthin is altered after section of the splanchnic nerves. Another difficulty in making deductions of a general nature lies in the fact that digitalis bodies differ in their action, in their solubility, and in the rate of their absorption. These difficulties are made clear when the following statements are placed side by side. Strophanthin is much more toxic to a frog's heart than digitonin, but the latter is more toxic to the isolated sartorius (40). Voegtlin and Macht (41), experimenting on isolated coronary arteries, found that digitonin and digalen produced relaxation of the

arteries, while digitoxin and digitalin caused constriction. Fraser (42), many years ago, called attention to the fact that while strophanthin was much more profound in its action on the frog's heart than digitalin, the reverse was true as to their action on the blood vessels.

There is also a marked difference in the reaction and susceptibility of different animals to these drugs. And the problem is further complicated by the change in the mode of administration from animal to animal. The intact grass snake, for instance, is 30 times more tolerant to strophanthin given subcutaneously than is the frog, but the isolated heart is 1,000 times as tolerant. The action of these drugs depends upon the state of the heart, on the temperature of the surrounding media, the proportion of inorganic salts in the fluid, the pressure to which the heart is exposed, the rate of the heart beat, the presence or absence of blood corpuscles or serum, and, no doubt, other factors as well. To obtain identical results under artificial conditions means that all these factors must be controlled. These are requirements which are obviously difficult to meet. The experiments now reported have avoided these difficulties.

The experiments have a bearing on the controversy between Straub and Grünwald. Straub believes that the action of strophanthin is independent of the total amount and depends entirely upon the concentration; Grünwald holds that although the concentration is the prime factor when large quantities of the active principle are used, the total amount is of importance when the dilutions are small and when small amounts are compared. The distinction is important because it is the small amounts and the low dilutions that are employed in clinical medicine. Furthermore, it is not safe to measure the toxicity of digitalis in terms of the time it takes to produce standstill of the ventricles, since Schmiedeberg (43) found that the two factors did not vary directly.

In these experiments, in which crystalline strophanthin was injected intravenously, the amount of the drug needed to produce toxic results as shown electrocardiographically was practically independent of the speed of administration. The use of the same animal for repeated experimentation avoided the error which re-

sults from making comparisons between different species of animals, and eliminated the marked variations due to individual susceptibility. Injections of the drug were made at varying speeds, with intervals of 4 or more days between experiments. The total amount required to produce the toxic effect did not vary significantly. The experiments (Table II) show strikingly that the total amount required to produce a toxic effect is independent of the speed of administration. This conclusion is probable because although the amount was the same, the concentration was considerably greater when 60 per cent of the minimum lethal dose was given in a single dose or in $\frac{1}{4}$ hour than when given in 4 hours. It has been suggested that when the drug is given slowly, it may remain inert in the blood stream and produce a toxic effect only when a proper concentration is reached, this concentration being reached when the required amount has been injected without reference to the speed of administration. Against this view we have noted the fact that the heart is known to be affected by the drug before toxic effects are produced. The transfusion experiments were undertaken to determine whether strophanthin was present in the blood stream. The method showed that strophanthin must be regarded as present and capable of remaining there for a moderate time but proved too crude to give an idea of its actual concentration. A theory of the action of strophanthin may be formulated from the above considerations. It reconciles the conflicting views relating to the importance of concentration and of total amount of the drug. It supposes that the time required to produce a given effect in a heart varies inversely with the concentration of the active principle. The heart utilizes only a small portion (in the neighborhood of 10 per cent) of the drug to which it is exposed no matter what the concentration. A toxic effect results when the heart has taken up a certain total amount of the drug which is a definite small fraction of its own weight. If this theory is correct, it explains why in concentrated solutions the total amount is not important, for the small part that is taken out by the heart does not appreciably alter the concentration, while when very dilute solutions or small quantities are used the amount taken up by the heart diminishes the remaining concentration appreciably;

that is, the "digitalis pressure" becomes lessened. In these experiments the rapid injections forced an adequate amount of strophanthin into the heart rapidly and produced the toxic effect; in the slow injections the same total amount of drug was taken up by the heart only more slowly.

Experiments were carried out on a series of cats in which, after the minimum toxic dose was determined, the injections (some were slow, others more rapid) were continued until death. In this way the margin of safety was determined, the difference between the minimum lethal dose and the minimum toxic dose. It varied from 6 to 42 per cent of the minimum lethal dose.

These experiments have an importance in clinical medicine. They suggest a method for intravenous medication with strophanthin, designed to reduce the danger due to using the drug. After the ventricles have fibrillated, there is no constant way of reviving a heart. Occasionally, as in one experiment, resuscitation may take place after ventricular fibrillation and standstill, by massage of the heart without opening the chest. No opinion can be offered on the practical value of this method.

CONCLUSIONS.

1. Cats vary considerably in their susceptibility to strophanthin and in the extent of the difference between the minimum lethal dose and the minimum toxic dose.

2. The amount of strophanthin necessary to produce a toxic effect in a given cat is independent of the speed of administration to a period of 4 hours. An improvement in the clinical administration of the drug is thereby indicated.

3. A theory of the action of strophanthin is formulated which reconciles the results which point to the importance of the total amount taken up by the heart with those which indicate that the concentration of the drug is the determining factor.

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EXPLANATION OF PLATES.

PLATE 23.

FIG. 1, *a*. Cat 12 d. This electrocardiogram was taken at 2.40 p.m. before strophanthin injections.

b. This curve was taken at 4.44 when 36 per cent of the minimum lethal dose had been given. It shows ventricular extrasystolic tachycardia, the typical toxic effect.

c. Curve taken at 5.43. 10 per cent in addition had been given at 5.42 (total of 46 per cent of the minimum lethal dose). It shows typical ventricular fibrillation.

FIG. 2, *a*. Cat 6 b. Curve taken at 3.35 p.m. before strophanthin was injected. It shows normal heart mechanism.

b. Curve taken at 4.13 after 35 per cent of the minimum lethal dose had been given. It shows a toxic effect in which the ventricular complex is continually changing. There are alterations also in the P–R relation.

PLATE 24.

FIG. 3, *a*. Cat 12 a. Curve taken at 4.57 p.m. before strophanthin injection. The first five contractions are nodal beats.

b. Curve taken at 5.05 after 20 per cent of the minimum lethal dose of strophanthin had been given. It shows a nodal rhythm; the auricular waves are lost in the ventricular complexes.

c. Curve taken at 5.07 after 28 per cent of the minimum lethal dose. It shows that the heart has returned to the normal mechanism.

PLATE 25.

FIG. 4, *a*. Cat 7 b. Curve taken at 12.15 p.m., before strophanthin was given. It shows normal rhythm.

b. Curve taken at 1.06 p.m., after 52 per cent of the minimum lethal dose had been given. It shows frequently changing ventricular complexes.

c. Curve taken at 1.15. No additional strophanthin. The curve shows ventricular fibrillation in the first portion and then a standstill of the heart.

d. Curve taken at 1.30 showing the gradual onset of a ventricular extrasystolic tachycardia.

e. Curve taken at 1.37 showing sequential contraction of auricles and ventricles but with delayed conduction; P−R = 0.16 second.

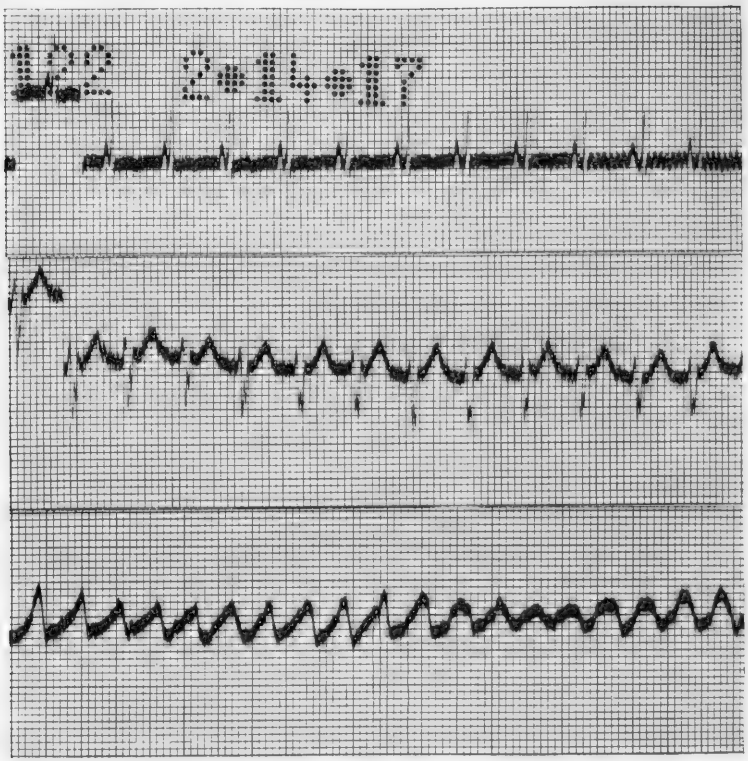


FIG. 1.

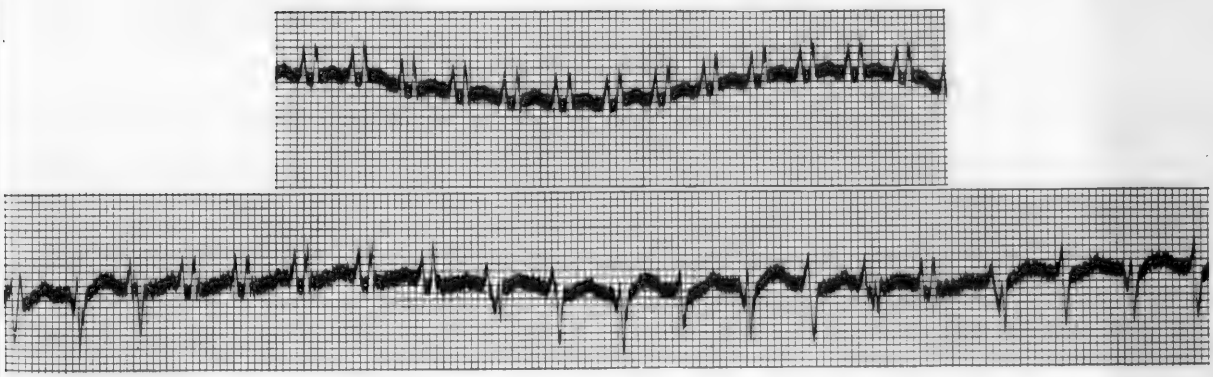


FIG. 2.

(Levine: Action of strophanthin on cat's heart.)

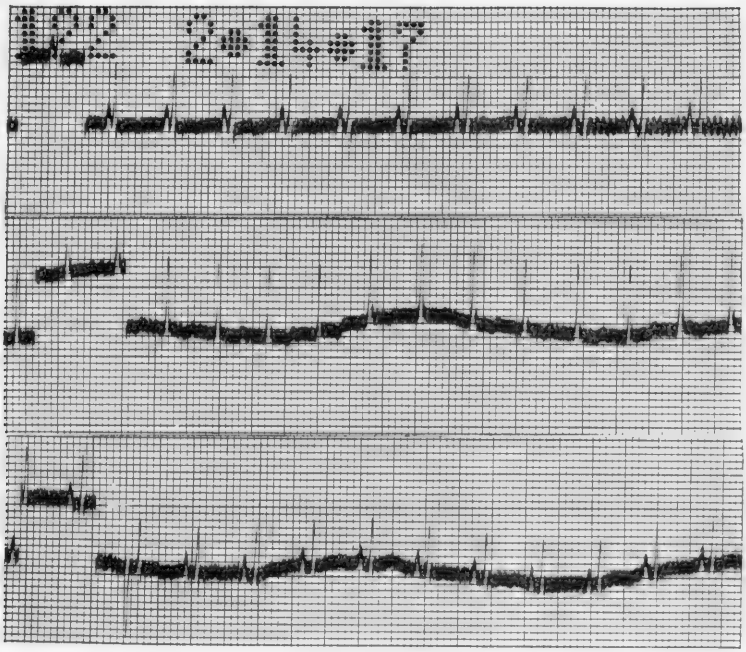
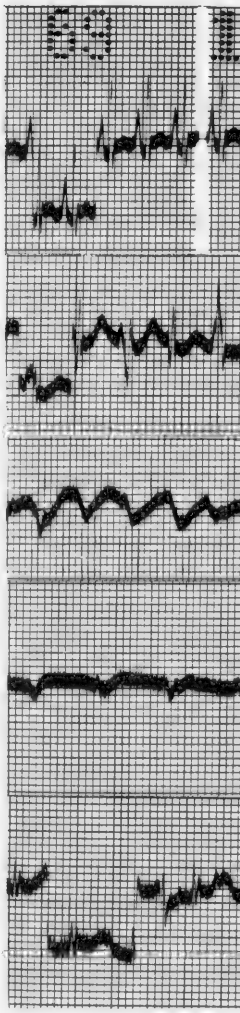


FIG. 3.

(Levine: Action of strophanthin on cat's heart.)



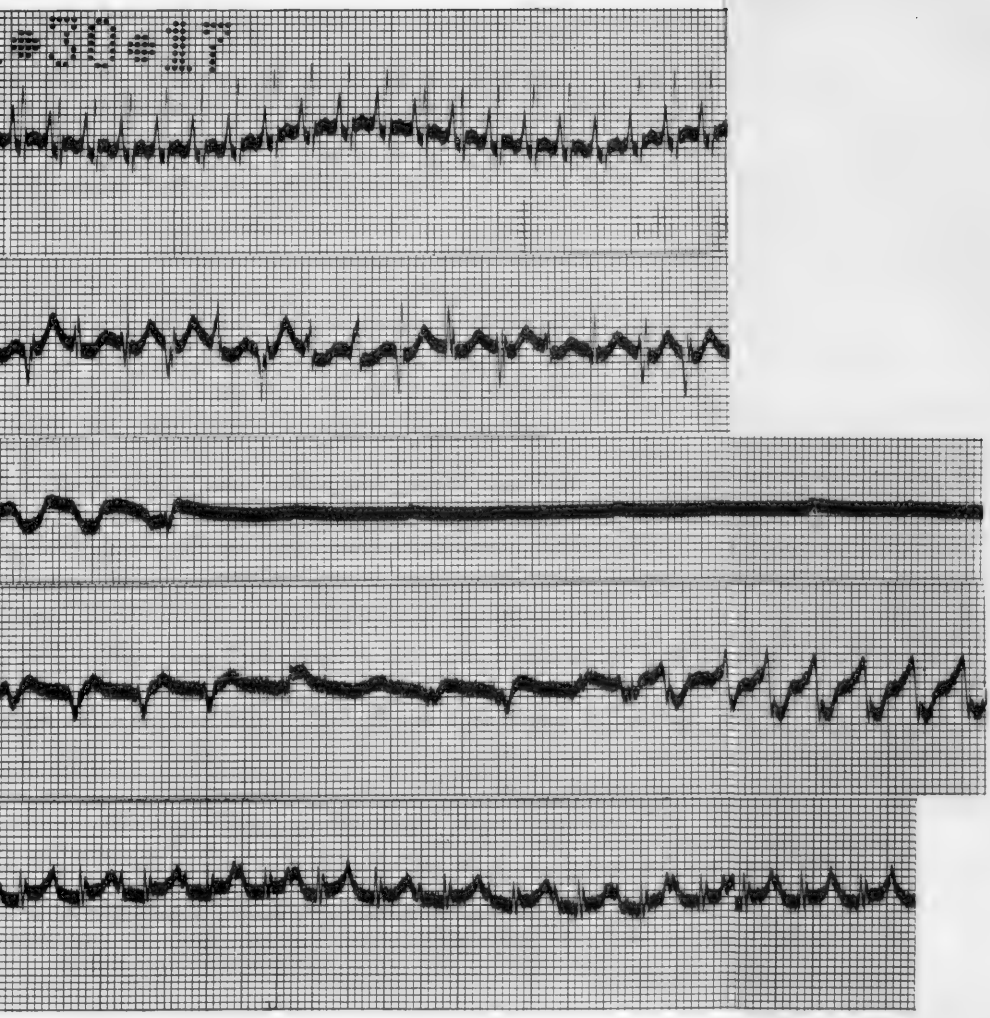


FIG. 4.

(Levine: Aion of strophanthin on cat's heart.)

A FUNCTIONAL AND PATHOLOGICAL STUDY OF THE CHRONIC NEPHROPATHY INDUCED IN THE DOG BY URANIUM NITRATE.*

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PLATES 26 TO 30.

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Since the initial observation in 1888 by Chittenden and Hutchinson[1] that uranium salts would induce an acute nephropathy, these substances have been extensively used as acute nephrotoxic agents. Only a few observations have been made of the effect of these salts on the kidney in prolonged intoxications. Such observations as have been made are largely concerned with the type of the pathological response on the part of the kidney, and a study of the processes of repair which take place in the kidney during its recovery from the acute injury.

In 1904 Richter[2] employed uranium to induce a condition in animals analogous to Bright's disease. As a result of Richter's observations, uranium was employed by Dickson[3,4] in two researches, in which he demonstrated that prolonged intoxications by uranium nitrate would induce in guinea pigs, rabbits, and dogs a kidney injury which was comparable with some of the types of chronic kidney disease found in man. The form of injury most frequently induced was

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[2] Richter, P. F., Die experimentelle Erzeugung von Hydrops bei Nephritis, *Beitr. klin. Med.*, Festschrift, Professor Senator, Berlin, 1904, 283.

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of a diffuse interstitial type which in some instances went to the stage of granular atrophy. Associated with these later stages, certain of the animals became polyuric.

The present investigation has been undertaken with the object in view of studying the functional capacity of the kidney during the period of acute injury from uranium, and also during the period when the kidney is recovering from the acute degeneration and passing into that stage of chronic injury which is characterized by such changes in structure that the kidneys may be considered to represent some type of chronic nephropathy. The investigation has a further object. In previous papers[5,6] the observation has been made that the acute injury to the normal kidney, or to the naturally nephropathic kidney, by uranium is associated with the development of an acid intoxication. In the present study observations will be made of the changes in the acid-base equilibrium of the blood of these animals, not only during the period of acute damage to the kidney, but during the period of recovery from such an injury, when the relation may be studied of such changes in the blood with the processes of repair in the kidney, and the return of the functional response of the kidney.

EXPERIMENTAL.

Twenty-seven female dogs have been used in this series of experiments. The animals have varied from 5 months to $10\frac{1}{2}$ years in age. They were kept in metabolism cages for 4 days prior to the commencement of the intoxication. During this period studies of the urine, blood, and the functional capacity of the kidney were made, in order to eliminate animals with a naturally acquired nephropathy. All the animals were free from renal disease. The animals were given 500 cc. of water daily, and fed on bread with

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a small amount of cooked lean beef. The animals were catheterized once a day, and the amount of urine obtained was added to the cage urine for analysis. The experiments were terminated at various periods during the intoxication without employing an anesthetic. This method of termination eliminates the probability of inducing acute degenerative changes in the liver as well as the kidney. The animals were poisoned by one dose of 4 mg. of uranium nitrate per kilo. The uranium was given subcutaneously.

During the course of the experiments the urine was examined quantitatively for albumin by Esbach's method, and for glucose with Benedict's reagent. The functional capacity of the kidney was studied by noting the percentage retention of blood urea, as shown by Marshall's[7] method, as modified by Van Slyke and Cullen,[8] and also by the elimination of phenolsulfonephthalein. The latter functional test was conducted according to the technique of Rowntree and Geraghty.[9] Observations on the acid-base equilibrium of the blood were made by employing the methods of Marriott[10,11] by ascertaining the alkali reserve of the blood and the tension of alveolar air carbon dioxide.

The Course of Prolonged Intoxication by Uranium in the Dog.

A large number of preliminary experiments has been necessary, in order to determine the dose of uranium which would effect a severe renal injury, and yet not be sufficiently toxic to injure the kidney so that processes of repair could not arise and lead to the development of a chronic nephropathy. As a result of these pre-

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[10] Marriott, W. McK., A method for the determination of the alkali reserve of the blood plasma, *Arch. Int. Med.*, 1916, xvii, 840.

[11] Marriott, W. McK., The determination of alveolar carbon dioxide tension by a simple method, *J. Am. Med. Assn.*, 1916, lxvi, 1594.

liminary experiments, an observation previously made by me[12] was confirmed, that the toxicity of uranium, as expressed by the severity of the kidney injury, largely depended upon the age of the animal.

A study of the experiments which follow not only illustrates the relative toxicity of uranium for animals of different ages, but also shows that young animals may so repair the damage done the kidney by uranium that they can, with the development of a chronic type of nephropathy, reestablish to some extent the function of the kidney, while in the older animals these changes of repair do not occur.

As a result of these observations it has seemed advisable to group the experiments used in this study according to the age of the animal. Group 1 is represented by seven animals between 6 and $10\frac{1}{2}$ years of age; Group 2, by eight animals varying from 1 to 4 years of age; while Group 3 includes twelve animals from 5 to 11 months of age. From the experiments in the different groups of animals, the course of the intoxication, as illustrated by one experiment typical for the group, is included in the form of a table. In order to reduce the space required for the tables, only observations on every 3rd day of the experiment have been tabulated.

Group 1. Animals Varying in Age from 6 to $10\frac{1}{2}$ Years.

Seven animals are included in this group. Four of the experiments were artificially terminated, one on the 6th, 8th, 10th, and 12th days respectively following the commencement of the uranium intoxication. Of the remaining three animals of the group two died on the 10th day of the intoxication in a comatose condition, which rapidly developed following a period of convulsions. The third animal died on the 6th day in air-hunger. None of the animals of this group survived the intoxication for longer than 12 days.

Reference to Table I shows the course of the intoxication in an animal representative of this group. During the first 24 hour period following the injection of uranium there occurs an increase

[12] MacNider, W. de B., A consideration of the relative toxicity of uranium nitrate for animals of different ages. I, *J. Exp. Med.*, 1917, xxvi, 1.

in the output of urine. At this early period both albumin and glucose appear in the urine, and the centrifugalized urine shows both hyaline and granular casts. Evidence of kidney injury is indicated by the sudden reduction in the elimination of phenolsulfonephthalein and by a retention of blood urea. In Experiment 1 the output of phenolsulfonephthalein was reduced within 24 hours from the normal elimination of 58 per cent to 20 per cent, while the blood urea showed a retention of 0.028 per cent as opposed to the normal of 0.015 per cent.

The acid-base equilibrium of the blood in this group of old animals has shown a similar abrupt change in favor of a decrease in the alkali reserve. The reserve alkali in Experiment 1 was reduced from 8.1 to 8, while the tension of alveolar air carbon dioxide was reduced from 41 mm. to 37 mm.

TABLE I.

Prolonged Uranium Intoxication. Group 1, Experiment 1.

Day of experiment.	Age and weight of animal.	Water in 24 hrs.	Uranium nitrate per kg.	Urine in 24 hrs.	Albumin.	Glucose.	Blood urea.	Phthalein.	R. pH.	Carbon dioxide tension.
		cc.	*mg.*	*cc.*	*gm.*	*per cent*	*per cent*	*per cent*		*mm.*
1	7 yrs. 12.35 kg.	500	0	471	0	0	0.015	58	8.1	41
2		500	4	760	1.3	0.9	0.028	20	8.0	37
5		500	0	419	4.0	1.75	0.040	Tr.	7.8	29
8		500	0	58	3.6	0.97	0.078	0	7.75	21

The progress of the intoxication in this group of old animals, as shown by Table I, has consisted in a rapid reduction in the formation of urine, an increase in the amount of both albumin and glucose in the urine, and a progressive decrease in the functional capacity of the kidney, as shown by the elimination of phenolsulfonephthalein and the retention of blood urea. This functional change in the kidney is associated with a rapid decrease in the alkali reserve of the blood and an associated reduction in the tension of carbon dioxide in alveolar air. Both the functional response of the kidney and the reduction in the alkali reserve of the blood are progressive in severity with the duration of the experiment. In

this group of old animals there has been no attempt at a restoration of renal function and no reestablishment of a normal acid-base equilibrium.

The kidneys from the animals of this group have shown no characteristic gross pathological change. The surface is smooth and the capsule has not been adherent. On section the cortex is pale. The tissue at the corticomedullary junction has shown a large amount of fat, which extends into the cortex in streaks. The histological study has shown a degree of degeneration which depends for its severity and extent upon the duration of the intoxication. In the animals of this group in which the kidneys were obtained prior to the 8th day, the degenerative changes have been largely confined to the tubules. These changes have consisted in an acute swelling and vacuolation, which first appears in the third portion of the convoluted tubule. This change is followed by pyknosis of the nuclei and necrosis of the epithelium. Even at this early stage of the intoxication the ascending limb of Henle's loops contains a large amount of fat. The accumulation of fat is more marked in the kidneys of this older group of animals than in any of the remaining younger groups.

By the 10th to the 12th days of the intoxication the epithelial injury has become more diffuse. All the proximal convoluted tubule has participated in the degeneration, and the epithelium of the distal convoluted tubules and junctional tubules has shown evidence of injury. In addition to the extension of the epithelial injury the vascular tissue of the kidney becomes involved in the degeneration. The intertubular and glomerular capillary endothelium has shown a peculiar hyaline type of change, and later becomes vacuolated. Following this degeneration of the vascular tissue exudates occur between the tubules and into the capsular space of the glomeruli (Fig. 1).

In this group of old animals there has been no indication of an attempt to repair the injury. There is no evidence of nuclear division.

Group 2. Animals Varying in Age from 1 to 4 Years.

Eight animals are included in this group. Three of the experiments were terminated on the 6th day and two on the 10th day. Of the remaining animals, one died on the 14th day and two on the 21st day.

The course of the intoxication in the animals of this age grouping resembles in general the response obtained with the older animals of Group 1.

The injection of the animals of this group with 4 mg. of uranium nitrate is followed within 24 hours by the appearance of both albumin and glucose in the urine (Table II). Following an initial increase in the formation of urine the daily output is rapidly reduced so that by

TABLE II.

Prolonged Uranium Intoxication. Group 2, Experiment 2.

Day of experiment.	Age and weight of animal.	Water in 24 hrs.	Uranium nitrate per kg.	Urine in 24 hrs.	Albumin.	Glucose.	Blood urea.	Phthalein.	R. pH.	Carbon dioxide tension.
		cc.	*mg.*	*cc.*	*gm.*	*per cent*	*per cent*	*per cent*		*mm.*
1	3 yrs. 14.7 kg.	500	0	400	0	0	0.015	68	8.0	42
2		500	4	413	0.5	0.61	0.015	63	7.95	37
5		500	0	634	0.7	1.25	0.018	5	7.8	27
8		500	0	450	1.3	1.87	0.023	Tr.	7.8	25
11		500	0	210	0.9	1.03	0.031	"	7.75	21
14		500	0	35	0.5	0.87	0.080	0	7.65	18

the 14th day, as indicated in Experiment 2, only 35 cc. of urine were formed. Within a few hours of the appearance of albumin in the urine there is a reduction in the reserve alkali of the blood, a decrease in the elimination of phenolsulfonephthalein, and a retention of blood urea. These changes in the functional capacity of the kidney and in the acid-base equilibrium of the blood progress in severity with the duration of the experiment.

A study of tables of animals representative of Groups 1 and 2 (Tables I and II) shows the following variations in the response of the animals of the two groups to the intoxication. The older animals of Group 1 show a more rapidly developing intoxication, as indicated by an earlier reduction in the formation of urine, by the urine's contain-

ing a larger amount of both albumin and glucose, and by the more rapid development of a severe kidney injury. In addition to these variations in the severity of the intoxication in the two groups of animals, a similar variation has occurred in the rate with which the alkali reserve of the blood in the two groups shows a depletion. By the 8th day of the intoxication in Experiment 1 of Group 1 the alkali reserve had been reduced to 7.75, while in Experiment 2 of the younger group of animals, Group 2, this degree in the reduction of the alkali reserve was obtained on the 11th day.

A study of the rate with which the kidney shows evidence of injury in the two groups indicates that the kidneys of the older animals are more susceptible to injury than is the case with the younger group of animals. In Experiment 1 of Group 1, within the first 24 hour period the elimination of phenolsulfonephthalein was reduced from 58 per cent to 20 per cent, and a retention of blood urea developed of 0.028 per cent as compared with the normal of 0.015 per cent.

In the younger animals of Group 2, as illustrated by Experiment 2, such a rapid injury to the kidney did not develop. During the first 24 hour period of the intoxication the elimination of phenolsulfonephthalein was only reduced from 68 per cent to 63 per cent, and during this same period there was no retention over the normal in blood urea.

The pathological study of the kidneys from the animals of Group 2 has shown only one outstanding difference from the pathological reaction described as occurring in the animals of Group 1. In both groups the epithelium of the convoluted tubules has shown the most marked evidence of injury. The type of injury has been the same. The kidneys of the younger group of animals, Group 2, that have been obtained after the 6th day of the intoxication, show definite evidence of repair by a beginning regeneration of tubular epithelium (Fig. 2). The regeneration first appears in the third or terminal portion of the convoluted tubule. In the cells of this portion of the tubule which have sufficiently withstood the process of degeneration, new epithelium is formed by a process of indirect cell division. At first the newly formed cells are large and have a clear cytoplasm. Later these cells flatten out and contain a small amount of deeply staining cytoplasm in proportion to the large hypochromatic nuclei.

In Fig. 2 the different stages in the regeneration of atypical cells in the convoluted tubules is clearly shown.

In this second group of animals which differ from the first group in that an attempt at repair has commenced in the kidneys, the processes of repair have not sufficiently progressed to be expressed by any improvement in the functional capacity of the kidney or by any change in the direction of restoring the normal acid-base equilibrium of the blood.

Group 3. Animals Varying in Age from 5 to 11 Months.

Twelve animals are included in this group. Two of the experiments were terminated on the 6th day of the intoxication, four on the 16th day, two on the 20th, two on the 35th day, and the remaining two experiments on the 48th day.

A study of Tables III and IV, of Group 3, shows not only the stage of degeneration in these animals as indicated by the kidney injury and the disturbance in the acid-base equilibrium of the blood, but also the second period in the intoxication, which is characterized by a partial restoration in the functional capacity of the kidney, and by an attempt to restore the normal acid-base equilibrium of the blood.

The response of this youngest group of animals to an intoxication by uranium is similar qualitatively to the response which has been obtained in Groups 1 and 2 of the older animals. The difference in the reaction of these younger animals to uranium is shown by a delay in the development of the kidney injury and by the fact that the alkali reserve of the blood does not show so rapid a depletion as was the case with the older groups of animals. A further study also shows that an animal of this group (Experiment 4, Table IV) may during the course of the intoxication show as marked evidence of kidney injury and as great a depletion in alkali reserve as did the older animals of Groups 1 and 2, yet following this degree of injury the younger animals (Group 3) are able to some extent to return to the normal, while with the older animals such a reparative reaction has not occurred.

In Experiments 3 and 4 (Tables III and IV) these younger animals have shown less albumin in the urine than has been the case with the older animals. During the first 24 hour period of the intoxication

these animals have shown a reduction in the elimination of phenolsulfonephthalein and a retention of blood urea. In these animals, however, the reduction in the elimination of the dye is not so rapid and the retention of blood urea is not so great as is the case with the older groups of animals.

In this group of young animals there is the usual association between the kidney injury and the reduction in the alkali reserve of the blood. This disturbance in the acid-base equilibrium of the blood shows a gradual development. In Experiment 4 (Table IV)

TABLE III.

Prolonged Uranium Intoxication. Group 3, Experiment 3.

Day of experiment.	Age and weight of animal.	Water in 24 hrs.	Uranium nitrate per kg.	Urine in 24 hrs.	Albumin.	Glucose.	Blood urea.	Phthalein.	R. pH.	Carbon dioxide tension.
		cc.	*mg.*	*cc.*	*gm.*	*per cent*	*per cent*	*per cent*		*mm.*
1	8 mos. 15.9 kg.	500	0	418	0	0	0.012	58	8.1	40
2		500	4	623	0.3	0.7	0.016	33	8.05	40
5		500	0	310	1.0	1.19	0.024	22	8.0	40
8		500	0	105	0.6	0.52	0.048	18	7.9	32
11		500	0	230	0.2	0.3	0.070	10	7.9	31
14		500	0	120	0.2	0.1	0.100	0	7.9	32
17	8 mos. 12.3 kg.	500	0	503	Tr.	0	0.200	0	7.9	34
20		500	0	700	0	0	0.206	Tr.	7.9	30
23		500	0	700	0	0	0.208	"	7.9	30
26		500	0	794	0	0	0.218	4	7.95	36
29		500	0	628	0	0	0.180	8	7.95	38
32		500	0	623	Tr.	0	0.188	10	7.95	38
35		500	0	629	0	0	0.163	10	8.0	38

the greatest reduction in alkali reserve was 7.75, which was obtained on the 12th day of the experiment. In Experiment 3 the greatest reduction was 7.9. This reading was first obtained on the 8th day of the intoxication.

Judging by the percentage elimination of phenolsulfonephthalein, the degree of retention of blood urea, and the maximum reduction in the alkali reserve of the blood, we find that the height of the intoxication by uranium in these animals is reached between the 8th and 15th days of the experiment. Following this period, in this group of young

animals, the functional capacity of the kidney shows a beginning improvement and the alkali reserve of the blood shows less depletion. The histological study of the kidney during such a period of functional recuperation shows, in addition to the evidence of repair in the connective tissue element, a progressive epithelial regeneration.

In Experiment 4, Group 3, (Table IV), the height of the intoxication was reached on the 15th day of the experiment. At this stage

TABLE IV.

Prolonged Uranium Intoxication. Group 3, Experiment 4.

Day of experiment.	Age and weight of animal.	Water in 24 hrs.	Uranium nitrate per kg.	Urine in 24 hrs.	Albumin.	Glucose.	Blood urea.	Phthalein.	R. pH.	Carbon dioxide tension.
		cc.	*mg.*	*cc.*	*gm.*	*per cent*	*per cent*	*per cent*		*mm.*
1	11 mos. 12.58 kg.	500	0	605	0	0	0.016	81	8.0	42
2		500	4	619	0.75	0.81	0.028	56	7.95	38
3		500	0	211	1.2	0.85	0.038	51	7.9	34
6		500	0	189	0.4	0.27	0.077	31	7.9	34
9		500	0	475	Tr.	Tr.	0.177	8	7.85	31
12		500	0	192	0	"	0.180	Tr.	7.75	20
15		500	0	397	0	0	0.297	0	7.75	24
18		500	0	653	Tr.	0	0.183	24	7.8	27
21		500	0	611	"	0	0.108	24	7.8	27
24	11 mos. 9.5 kg.	500	0	459	Tr.	0	0.073	29	7.8	27
27		500	0	561	"	0	0.082	35	7.85	30
30		500	0	355	0.25	0	0.045	37	7.95	37
33		500	0	283	0.6	0	0.120	21	7.9	36
36		500	0	523	0.7	0.34	0.054	21	7.95	36
39		500	0	525	0.2	0	0.061	16	8.0	40
42		500	0	610	0.2	0	0.082	21	8.0	40
45		500	0	531	Tr.	0	0.053	28	8.0	40
48		500	0	566	"	0	0.065	28	8.0	40

of the experiment the animal gave a negative phenolsulfonephthalein test, the blood urea showed a retention of 0.297 per cent, and the alkali reserve of the blood had been reduced to 7.75. On the 18th day the animal had an elimination of phenolsulfonephthalein of 24 per cent, a reduction in the retention of blood urea to 0.183 per cent, and an increase in the alkali reserve of the blood to 7.8. During the further course of the experiment, which was terminated on

the 48th day, there occurred a gradual increase in the elimination of phenolsulfonephthalein, a decrease in the retention of blood urea, and an increase in the alkali reserve of the blood until the 30th day of the intoxication. At this period there occurred an unexplained relapse in the animal's condition. The phenolsulfonephthalein elimination dropped to 21 per cent and during the following 6 days went as low as 16 per cent. With this change in the ability of the kidney to eliminate the dye, the blood urea rose from a retention of 0.045 per cent to a retention of 0.120 per cent. The alkali reserve of the blood was reduced from 7.95 to 7.9. Following this period of secondary injury another phase of improvement developed, so that at the termination of the experiment on the 48th day the animal had an elimination of phenolsulfonephthalein of 28 per cent, a blood urea of 0.065 per cent, and a reserve alkali reading of 8.0.

The second experiment representative of this group of young animals (Experiment 3, Table III) shows a type of response similar to that of Experiment 4, which has been discussed in detail. Experiment 3 was terminated on the 35th day of the intoxication, 6 days after the functional response of the kidneys gave evidence of an improvement in the degree of intoxication and after the beginning restoration of the acid-base equilibrium of the blood.

The pathology of the kidney in the experiments of Group 3 has been studied at two periods of the intoxication. Experiments have been terminated and the kidneys obtained for study within 10 days after the functional observations showed the period of improvement in the animals to have been established. In other animals of the group the experiments were terminated after the stage of improvement had persisted for a longer period, 20 to 38 days. By such a selection of tissue not only have observations been made of the processes of repair in the kidney, but it has been possible to study these changes in connection with the reestablishment of the functional capacity of the kidney.

The kidneys obtained from animals in the early stages of improvement from the intoxication show no gross evidence of disease.

The histological study shows a late stage in the regeneration of tubular epithelium (Fig. 3). The regenerated epithelium remains of the flattened atypical type. The cytoplasm which stains deeply has

differentiated into cell units. The nuclei are hyperchromatic and of large size in proportion to the surrounding cytoplasm. Most of the granular detritus, the result of the early epithelial necrosis, has been removed from the newly lined tubules. In some of the tubules regeneration has failed to develop. Such tubules are filled with necrotic material. A diffuse formation of intertubular connective tissue has occurred which is in the cellular stage.

The vascular pathology has consisted in an invasion of the glomeruli by fibroblasts with a matting together of the capillary loops. The capsules of the glomeruli have shown an early thickening with a marked periglomerular cellular fibrosis (Fig. 4).

The kidneys obtained from animals late in the period of recovery from the intoxication have failed to show any gross change, such as an adherent capsule, scarring of the cortex, or any marked decrease in the proportionate relation between the cortex and medulla.

The histological study of this tissue has shown a later stage in the process of repair than was shown by tissue obtained from animals soon after a beginning recovery from the intoxication had been established. The sections have shown an increase in the number of tubules containing flattened, regenerated epithelium. Tubules with degenerating and necrotic epithelium are less numerous. The glomeruli show a later stage of fibrosis and the capsules show newly formed connective tissue fibers. The periglomerular and intertubular connective tissue has passed from the cellular stage to the stage of laying down connective tissue fibers (Fig. 5).

DISCUSSION.

The experiments conducted in this investigation confirm the earlier work of Dickson, who demonstrated that uranium would produce in certain of the lower animals a chronic kidney injury comparable with certain of the chronic diffuse nephropathies of man. The experiments furthermore demonstrate the character and the severity of the functional disturbance associated with the various stages of the uranium intoxication. In these experiments it has been shown that the severity of the acute degenerative changes in the kidney is largely dependent upon the age of the animal. The older

animals develop a more rapid and a severer type of intoxication than is the case with the younger animals. The intoxication is characterized by a reduction in the alkali reserve of the blood and by the development of a kidney injury. The injury to the kidney is expressed functionally by the appearance of albumin in the urine, a reduction in the elimination of phenolsulfonephthalein, and by a retention of blood urea. The reduction in the elimination of the dye occurs as the first indication of renal injury. The reduction progressively increases with the severity of the degeneration in the kidney. The retention of blood urea is a somewhat later manifestation of kidney injury. A retention of blood urea may be deferred for 6 to 36 hours after the development of a marked reduction in the ability of the kidney to eliminate phenolsulfonephthalein. Subsequently, during the period of acute degeneration, the retention of blood urea shows a progressive increase.

Albumin has appeared in the urine of all the animals during the first 24 hours. Following its appearance the amount of albumin increases for the first 2 to 6 days of the experiment, and during this period the quantitative output of albumin has shown a relation with the renal functional tests. After this period, however, the amount of albumin in the urine rapidly decreases, while the reduction in the elimination of phenolsulfonephthalein and the retention of blood urea progressively increase. From this observation it would appear that quantitative determinations of the amount of albumin in the urine may give very imperfect information concerning the degree of renal injury.

All the animals intoxicated by uranium have shown a disturbance in the acid-base equilibrium of the blood, as indicated by a reduction in the alkali reserve and by a decrease in the tension of alveolar air carbon dioxide. The depletion in the alkali reserve has developed more rapidly and has shown a greater degree of reduction early in the experiments on the older animals than has been the case with the younger animals. The severity of the intoxication, as expressed by the degree of functional disturbance of the kidneys, has shown a parallel with the severity of the disturbance in the acid-base equilibrium of the blood.

A study of the intoxication in the animals of the various age groups shows that the older animals develop a type of kidney injury in which there is no attempt at regeneration, and therefore no reestablishment of renal function. In these animals there has failed to develop any attempt to restore the normal acid-base equilibrium of the blood. In the younger animals, however, epithelial regeneration does occur, and following this attempt at repair the functional capacity of the kidney improves and the depletion of the alkali reserve of the blood is in part restored. The earliest evidence of such functional restoration has consisted in a return of the ability of the kidney to eliminate phenolsulfonephthalein. Following this improvement the percentage retention of blood urea is decreased and still later the acid-base equilibrium of the blood returns towards the normal. In two of the younger animals during the period of recovery from the intoxication, the acid-base equilibrium of the blood was restored to the point of normality, yet in these animals the retention of blood urea remained high and the elimination of phenolsulfonephthalein was sufficiently low to indicate the existence of a severe grade of kidney injury.

CONCLUSIONS.

1. Uranium nitrate is relatively more toxic for old animals than for young animals.

2. This relative toxicity is not only expressed in the old animals by a greater functional disturbance of the kidney, but is also shown by an inability on the part of these animals to repair the kidney injury and reestablish its functional capacity.

3. The intoxication in younger animals has been followed by a repair of the renal injury and a partial restoration of kidney function.

4. In these animals the processes of repair lead to the development of a chronic diffuse type of nephropathy in which the acid-base equilibrium of the blood may be maintained at the point of normality. In these animals renal functional tests indicate the presence of severe kidney injury.

EXPLANATION OF PLATES.

PLATE 26.

FIG. 1. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the animal of Experiment 1, Group 1 (Table I). It shows at *A* an acute swelling and vacuolation of the convoluted tubule epithelium. At *B* are shown tubules in which the epithelial degeneration has advanced to the stage of complete necrosis. At *C* is shown a junctional tubule distended with necrotic epithelial debris. Between the convoluted tubules at *D* there has occurred an extravasation of blood.

PLATE 27.

FIG. 2. Camera lucida drawing, Leitz oc. 2, obj. $\frac{1}{12}$. Oil immersion. The figure is from the kidney of the animal of Experiment 2, Group 2 (Table II). It represents the different stages in the regeneration of a flattened atypical type of convoluted tubule epithelium. At *A* is shown a regenerated epithelial cell containing a mitotic figure. This tubule is partially lined by regenerated cells. The tubule also contains the remains of necrotic cell material. At *B* and *C* are shown tubules in the early stage of epithelial regeneration. At *D* is shown a tubule in a later stage of the process of epithelial regeneration. The large epithelial cells which are first regenerated are here flattening out to line the tubule with an atypical and less specialized type of epithelium, which is characterized by large hyperchromatic nuclei and a small amount of deeply staining cytoplasm.

PLATE 28.

FIG. 3. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the animal of Experiment 3, Group 3 (Table III). It shows a later stage in the regeneration of tubular epithelium and the stage in the delayed uranium intoxication which is characterized by the formation of intertubular connective tissue. At *A* are shown convoluted tubules lined by a flattened type of epithelium. The cytoplasm has differentiated with the formation of definite cell boundaries. The necrotic material has been removed from the lumen of the tubules. At *B* are shown tubules filled with the remains of necrotic epithelium. No epithelial regeneration has developed in these tubules. At *C* is shown the early formation of cellular connective tissue.

PLATE 29.

FIG. 4. Camera lucida drawing, Leitz oc. 2, obj. 6. This figure is taken from the kidney of the same animal as Fig. 3. It shows at *A* a glomerulus in an early stage of connective tissue infiltration. Connective tissue nuclei are numerous. The capillary loops have become obliterated and matted together. At *B* is shown an early thickening of the capsule of the glomerulus and a marked peri-

glomerular cellular fibrosis. At *C* are shown tubules lined with regenerated flattened epithelium. At *D* convoluted tubules may be seen in an early stage of degeneration. At *E* is shown a marked intertubular cellular fibrosis in which are several newly lined tubules.

PLATE 30.

FIG. 5. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the animal of Experiment 4, Group 3 (Table IV). It shows an advanced stage of repair following the uranium intoxication which has resulted in the development of a chronic diffuse type of nephropathy. At *A* is shown the predominance of regenerated tubules, lined by flattened epithelium. At *B* is shown a shrunken glomerulus with a capsule thickened by the laying down of connective tissue fibers. At *C* the intertubular connective tissue has passed from the early cellular stage into the stage of connective tissue fiber formation.

FIG. 1.

(MacNider: Chronic nephropathy by uranium nitrate.)

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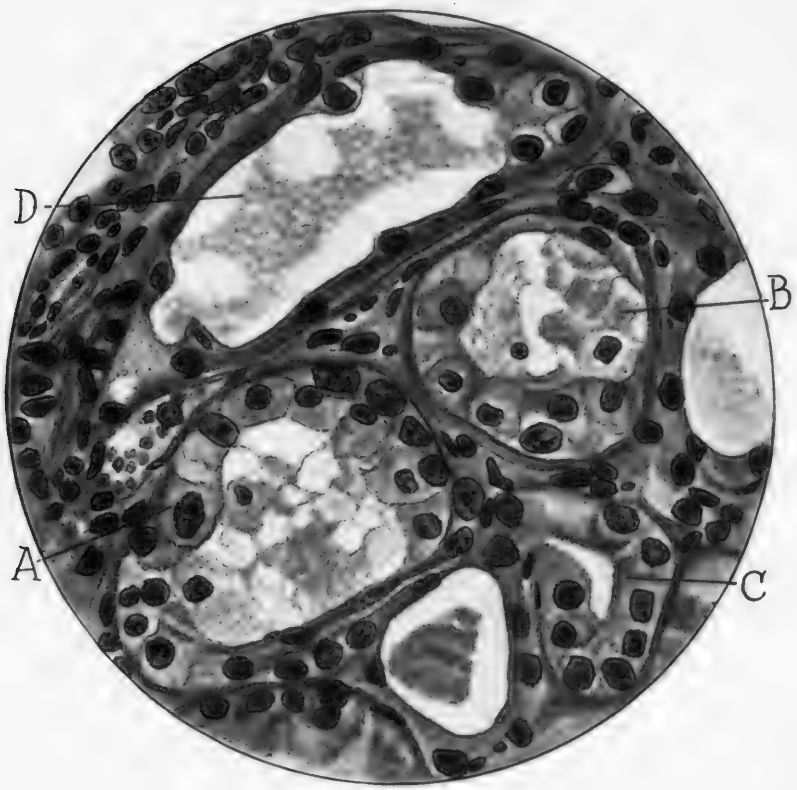

FIG. 2.

(MacNider: Chronic nephropathy by uranium nitrate.)

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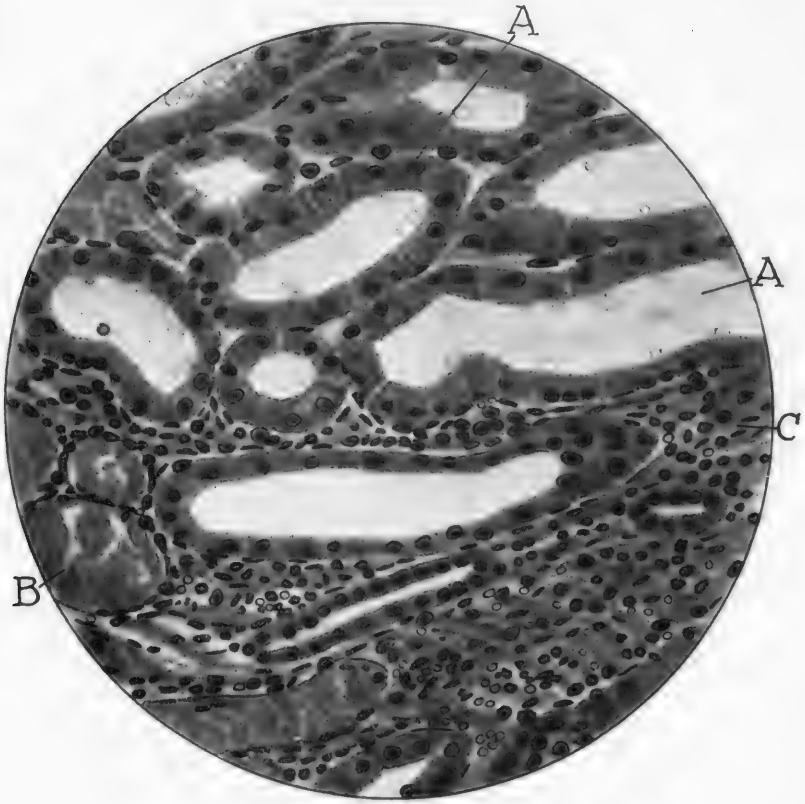

FIG. 3.

(MacNider: Chronic nephropathy by uranium nitrate)

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FIG. 4.

(MacNider: Chronic nephropathy by uranium nitrate.)

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FIG. 5.

(MacNider: Chronic nephropathy by uranium nitrate.)

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